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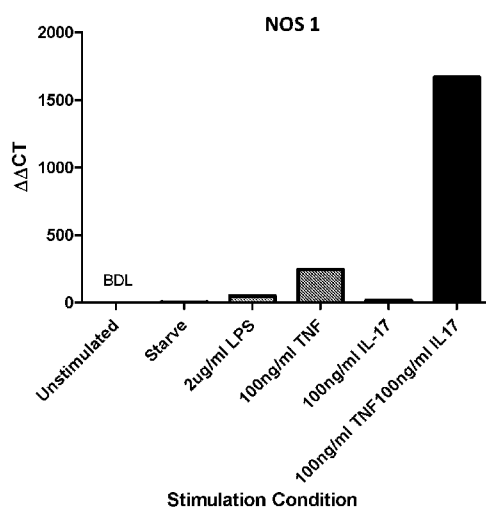
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[Continued on next page]

- (54) **Title:** COMPONENTS OF THE UREA CYCLE AS BIOMARKERS FOR INFLAMMATORY DISEASE AND METHODS OF USING SAME



B

Figure 6

- (57) **Abstract:** The invention provides methods for predicting the efficacy of anti-TNF and anti-IL-17 combination therapies in the treatment of a subject suffering from inflammatory disease by determining the abundance of a biomarker associated with the urea cycle in a sample derived from the subject.



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COMPONENTS OF THE UREA CYCLE AS BIOMARKERS FOR INFLAMMATORY DISEASE AND METHODS OF USING SAME

5

REFERENCE TO RELATED APPLICATIONS

This international application claims the priority of U.S. provisional application serial number 62/183,102, filed June 22, 2015, which is incorporated by reference herein in its entirety.

10

BACKGROUND OF THE INVENTION

Anti-cytokine therapies have become the standard of care for treating the symptoms and arresting the disease progression of inflammatory diseases. But despite the numerous treatment options, many patients still fail to experience a substantial decrease in disease activity. In principle, increasing the level of immunosuppression by combining agents is a plausible strategy for achieving improved efficacy. But attempts to combine anti-cytokine therapies to this end have been plagued by unacceptable safety and tolerability issues. Nevertheless, finding a combination therapy for the treatment of inflammatory disease that provides both an improved response and acceptable safety remains a challenge.

20

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease with unknown etiology. Its primary organ manifestations include joint inflammation resulting in pain, swelling and progressive bone and cartilage destruction, with numerous co-morbidities that include anemia and increased risk of cardiovascular events. As of 2012, over 5 million people were afflicted with RA, with approximately 26% having mild, 49% moderate, and 25% severe disease, with women being affected three (3) times more than men. In many cases, current treatment regimens are not completely efficacious.

Anti-tumor necrosis factor (TNF) therapies are the most prescribed anti-cytokine therapies for RA. TNF is a pro-inflammatory cytokine that triggers the acute phase response and increases expression of many mediators of pain, inflammation and joint destruction including other inflammatory cytokines and matrix metalloproteases and activate several pathways, including the NF- κ B, MAPK, and apoptosis pathways. In many RA patients that fail to achieve remission, and in rodent disease models, anti-TNF therapy is only partially effective in suppressing the effects of this pro-inflammatory

cytokine. Based on a number of in vitro studies, TNF appears to cooperate with IL-17 in regulating pro-inflammatory gene expression, making the dual anti-TNF/anti-IL-17 treatment an attractive combination therapy.

It remains to be seen whether the dual inhibition of TNF and IL-17 will be safe and effective in all patients. Biomarkers are typically used as measurable indicators of disease severity or progression, and to evaluate the most effective therapeutic regimen for the treatment of diseases. Biomarkers in the context of drug development include changes in the expression patterns of certain gene products, such as an increase or decrease in the level of a certain protein in the serum. In particular, biomarkers can be used to predict whether a drug will be effective in a particular patient or patient population and to tailor a patient's treatment options. Whereas a number of biomarkers are available to the clinician as a general indicator of inflammation, the efficacy of, or response to, certain anti-inflammatory treatments can be indicated by a particular biomarker(s).

Accordingly, there is a need in the art for measurable indicators of drug efficacy as well as methods for assessing or predicting responsiveness to combined inflammatory disease therapies comprising anti-TNF and anti-IL-17.

SUMMARY OF THE INVENTION

The instant description provides a method of monitoring or calibrating a dosage in a subject being treated for rheumatoid arthritis with a combination therapy comprising an anti-TNF treatment and an anti-IL-17 treatment. The method comprises the steps of administering to the subject a first dose of a combination therapy comprising an anti-TNF treatment and an anti-IL-17 treatment; determining a modulation of expression of one or more biomarkers in a sample from the subject, wherein the one or more biomarkers are associated with the urea cycle; and administering a second dose of the combination therapy, wherein the second dose is determined depending on the relative abundance of the one or more biomarkers in the subject sample in response to the first dose.

In certain embodiments, the biomarkers are selected from the group consisting of arginine, arginase II, argininosuccinate lyase, nitric oxide synthetase (NOS) 1, and argininosuccinate synthetase 1. In one aspect of this embodiment, the determination of modulation of expression of the one or more biomarkers in the sample from the subject

comprises detecting the interaction of one or more binding moieties that specifically bind to the one or more gene products or nucleic acids expressing the gene products, thereby detecting the abundance of the one or more biomarkers in the subject sample; and obtaining a relative abundance of the one or more biomarkers in the subject sample
5 by comparison to a baseline abundance of the biomarker.

In various embodiments, the baseline abundance of the one or more biomarkers is detected prior to administering to the subject the first dose of the combination therapy. For example, the baseline abundance is detected hours, days or months prior to administering the first dose. In any of the embodiments described herein, the baseline
10 abundance of the one or more biomarkers is the abundance of the biomarker in a healthy subject, who does not have rheumatoid arthritis.

In another embodiment, the second dose is equal to or greater than the first dose when the one or more biomarkers are selected from the group consisting of arginase II, argininosuccinate lyase, and NOS1, and wherein the relative abundance of the one or
15 more biomarkers in the subject sample in response to the first dose is greater when compared to the baseline abundance of the one or more biomarkers.

In another embodiment, the second dose is equal to or greater than the first dose when the one or more biomarkers is argininosuccinate synthetase 1 and wherein the relative abundance of the argininosuccinate synthetase 1 in the subject sample in
20 response to the first dose is less when compared to the baseline abundance of the argininosuccinate synthetase 1.

In another embodiment, the second dose is less than the first dose or treatment is discontinued when one or more biomarkers are selected from the group consisting of arginase II, argininosuccinate lyase, and NOS 1, and wherein the relative abundance of
25 the one or more biomarkers in the subject sample in response to the first dose is less when compared to the baseline abundance of the one or more biomarkers.

In another embodiment, the second dose is less than the first dose or treatment is discontinued when one or more biomarkers is argininosuccinate synthetase 1 and wherein the relative abundance of the argininosuccinate synthetase 1 in the subject
30 sample in response to the first dose is greater when compared to the baseline abundance of the argininosuccinate synthetase 1.

In another embodiment, the second dose is equal to or greater than the first dose when the one or more biomarkers is arginine, and wherein the relative abundance of the

arginine in the subject sample in response to the first dose is greater when compared to the baseline abundance of arginine.

In another embodiment, the second dose is less than the first dose or treatment is discontinued when one or more biomarkers is arginine, and wherein the relative
5 abundance of the arginine in the subject sample in response to the first dose is less when compared to the baseline abundance of arginine.

The instant description also provides a method of treating a subject suffering from rheumatoid arthritis, the method comprising the steps of administering a dose of a combination therapy comprising an anti-TNF treatment and an anti-IL-17 treatment to
10 the subject, wherein a sample from the subject comprises an abundance of one or more biomarkers, wherein the one or more biomarkers are selected from the group consisting of arginase II, argininosuccinate lyase and NOS 1, and wherein the relative abundance of the one or more biomarkers in the subject sample is greater when compared to a baseline abundance of the one or more biomarkers.

15 The instant description also provides a method of treating a subject suffering from rheumatoid arthritis, the method comprising the steps of administering a dose of a combination therapy comprising an anti-TNF treatment and an anti-IL-17 treatment to the subject, wherein a sample from the subject comprises an abundance of arginine, and wherein the relative abundance of arginine in the subject sample is greater when
20 compared to a baseline abundance of arginine.

The instant description also provides a method of treating a subject suffering from rheumatoid arthritis, the method comprising the steps of administering a dose of a combination therapy comprising an anti-TNF treatment and an anti-IL-17 treatment to the subject, wherein a sample from the subject comprises an abundance of
25 argininosuccinate synthetase 1, and wherein the relative abundance of argininosuccinate synthetase 1, in the subject sample is less when compared to a baseline abundance of the argininosuccinate synthetase 1.

The instant description also provides a method of screening a subject for rheumatoid arthritis and/or a method of determining modulation of expression of one or
30 more biomarkers in a subject having rheumatoid arthritis. In various embodiments, the method comprises the steps of determining a modulation of expression of one or more biomarkers in a sample from the subject, wherein the one or more biomarkers are selected from the group consisting of arginase II, argininosuccinate lyase, NOS 1, and

argininosuccinate synthetase 1; detecting the interaction of one or more binding moieties that specifically bind to the one or more gene products or nucleic acids expressing the gene products, thereby detecting the abundance of the one or more biomarkers in the subject sample; and obtaining a relative abundance of the one or more biomarkers in the
5 subject sample by comparison to a baseline abundance of the one or more biomarkers; wherein the subject has an increased risk of an inflammatory disorder when the abundance of the one or more biomarkers is modulated.

In any of one or more of the embodiments described herein, the baseline abundance of the biomarker can be the abundance of the biomarker in a healthy subject.
10 In any of one or more of the embodiments described herein the healthy subject is not experiencing rheumatoid arthritis. In any of the embodiments described herein, the baseline abundance of the biomarker can be the average abundance of the biomarker in two or more healthy subjects. In any of the embodiments described herein, the baseline abundance of the biomarker can be the abundance of the biomarker in the treated subject
15 before the subject experienced rheumatoid arthritis. In any of the embodiments described herein, the baseline abundance of the biomarker can be the abundance of the biomarker in the treated subject before the subject was experiencing symptoms of rheumatoid arthritis. In any of the embodiments described herein, the baseline abundance of the biomarker can be the abundance of the biomarker in the treated subject
20 when the subject's rheumatoid arthritis is in remission.

In any of the embodiments described herein, the sample can comprise joint tissue. In certain embodiments, the joint tissue comprises synovium tissue or synovial fluid. In certain embodiments, the synovium tissue comprises fibroblast-like synoviocytes.

25 The instant description also provides a method of decreasing the abundance of arginine in the serum of a subject comprising administering to the subject an effective amount of a combination therapy comprising an anti-TNF treatment and an anti-IL-17 treatment.

The instant description also provides a kit comprised of one or more binding
30 moieties that specifically bind to one or more gene products or nucleic acids expressing the gene products selected from the group consisting of arginase II, argininosuccinate lyase, NOS 1 and argininosuccinate synthetase 1. In certain embodiments, the kit comprises two or more binding moieties. In other embodiments, the kit includes means

for the detection of the abundance of arginine.

In various embodiments described herein, the anti-TNF treatment comprises an anti-TNF binding protein. For example, the anti-TNF treatment includes anti-TNF- α treatment. In various embodiments of the method, the anti-TNF binding protein
5 comprises a fusion protein, an antibody, or antigen binding fragment thereof that specifically binds to TNF. In various embodiments, the anti-TNF binding protein comprises a murine antibody, a human antibody, a humanized antibody, a bispecific antibody, a chimeric antibody, a Fab, a Fab', a F(ab')₂, an ScFv, an SMIP, an affibody, an avimer, a versabody, a nanobody, a domain antibody, or an antigen binding fragment
10 thereof.

In various embodiments of the method, the human anti-TNF- α antibody comprises Adalimumab, or an antigen binding fragment thereof. In various embodiments of the method, the anti-TNF antibody comprises a humanized anti-TNF antibody. For example, the humanized anti-TNF antibody comprises infliximab, or an
15 antigen binding fragment thereof. In various embodiments of the method, the anti-TNF binding protein comprises an anti-TNF- α fusion protein. For example, the anti-TNF- α binding protein comprises etanercept, or an antigen binding fragment thereof.

In various embodiments described herein, the anti-IL-17 treatment comprises an anti-IL-17 binding protein. In various embodiments of the method, the anti-IL-17
20 binding protein comprises a fusion protein, an antibody, or an antigen binding fragment thereof that specifically binds to IL-17. For example, the anti-IL-17 binding protein comprises a murine antibody, a human antibody, a humanized antibody, a bispecific antibody, a chimeric antibody, a Fab, a Fab', a F(ab')₂, an ScFv, an SMIP, an affibody, an avimer, a versabody, a nanobody, a domain antibody, or an antigen binding fragment
25 thereof.

For example, the anti-IL-17 antibody is ixekizumab, 10F7, B6-17, or an antigen binding fragment thereof.

The combination treatment in various embodiments of the methods described herein further comprises methotrexate, an analog thereof, or a pharmaceutically
30 acceptable salt thereof. In various embodiments of the method, the combination therapy comprises the administration of a multispecific binding protein that binds at least one of TNF and IL-17. For example, the multispecific binding protein is selected from the group consisting of a dual variable domain immunoglobulin (DVD-Ig) molecule, a half-

body DVD-Ig (hDVD-Ig) molecule, a triple variable domain immunoglobulin (TVD-Ig) molecule, a receptor variable domain immunoglobulin (rDVD-Ig) molecule, a polyvalent DVD-Ig (pDVD-Ig) molecule, a monobody DVD-Ig (mDVD-Ig) molecule, a cross over (coDVD-Ig) molecule, a blood brain barrier (bbbDVD-Ig) molecule, a

5 cleavable linker DVD-Ig (clDVD-Ig) molecule, and a redirected cytotoxicity DVD-Ig (rcDVD-Ig) molecule. In various embodiments of the methods described herein, the multispecific binding protein binds both TNF- α and IL-17. For example, the binding protein comprises a protein described herein, for example a DVD-Ig binding protein in Table 1, Table 2, or Table 17. In various embodiments, the DVD-Ig protein comprises

10 at least one variable heavy chain domain selected from Table 1, Table 2, or Table 17. In various embodiments, the DVD-Ig protein comprises at least one variable heavy chain domain selected from the group consisting of: SEQ ID NO: 5, SEQ ID NO: 11, and SEQ ID NO: 21. In various embodiments, the DVD-Ig protein comprises at least one variable light chain domain selected from Table 1, Table 2, or Table 17. In various

15 embodiments, the DVD-Ig protein comprises at least one variable light chain domain selected from the group consisting of: SEQ ID NO: 8, SEQ ID NO: 16, and SEQ ID NO: 26. In various embodiments, the combination therapy comprises a multispecific binding protein that binds TNF and IL-17 and comprises at least one of: a heavy chain amino acid sequence selected from SEQ ID NOs: 5, 11 and 24; a light chain amino acid

20 sequence selected from SEQ ID NOs: 8, 16, and 26; a heavy chain constant region selected from SEQ ID NOs: 7, 15, and 25; or a light chain constant region selected from SEQ ID NOs: 10, 20 and 30.

In various embodiments of any of the methods described herein, the one or more binding moieties specifically bind nucleic acids. In various embodiments of the

25 method(s), the one or more binding moieties specifically bind RNA. In various embodiments of the method, the one or more binding moieties specifically bind mRNA, miRNA, or hnRNA. In various embodiments of the method(s), the one or more binding moieties specifically bind DNA. In various embodiments of the method(s), the one or more binding moieties specifically bind cDNA.

30 In various embodiments of the method(s), the one or more binding moieties are appropriate for use in a technique selected from the group consisting of a polymerase chain reaction (PCR) amplification reaction, reverse-transcriptase PCR analysis, quantitative reverse-transcriptase PCR analysis, Northern blot analysis, an RNAase

protection assay, digital RNA detection/ quantitation, and a combination or sub-combination thereof.

In various embodiments of the method(s), the subject is a mammalian subject. For example, the mammal is selected from the group consisting of a human, a mouse, a rat, a non-human primate, a dog, a cat, a rabbit, a sheep, a goat and a pig. In various
5 embodiments of the method(s), the mammal is a human.

In various embodiments, the one or more binding moieties specifically bind a protein.

In various embodiments of the kit, the one or more binding moieties are binding
10 proteins that bind at least one of arginase II, argininosuccinate lyase, NOS 1 and argininosuccinate synthetase 1 protein or nucleic acid, or a homolog, portion or derivative thereof. For example, the one or more binding proteins comprise an antibody, or antigen binding fragment thereof, that specifically binds to the protein. In various
15 embodiments of the kit, the antibody or antigen binding fragment thereof is selected from the group consisting of a murine antibody, a human antibody, a humanized antibody, a bispecific antibody, a chimeric antibody, a Fab, a Fab', a F(ab')₂, an scFv, an SMIP, an affibody, an avimer, a versabody, a nanobody, a domain antibody, and an antigen binding fragment thereof.

In other embodiments, the baseline abundance of the biomarker is the abundance
20 of the biomarker in a healthy subject. In certain embodiments, the healthy subject is not experiencing the inflammatory disorder. In certain embodiments, the baseline abundance of the biomarker is the average abundance of the biomarker in two or more healthy subjects. In certain embodiments, the baseline abundance of the biomarker is the abundance of the biomarker in the treated subject before the subject experienced the
25 inflammatory disorder. In certain embodiments, the baseline abundance of the biomarker is the abundance of the biomarker in the treated subject before the subject was experiencing symptoms of the inflammatory disorder.

BRIEF DESCRIPTION OF THE DRAWINGS

30 Figure 1, panel A is a protocol for a mouse collagen induced arthritis (CIA) model involving injecting collagen II and complete Freund's adjuvant (CFA) into subjects at day zero. For one group, subjects were either administered a prophylactic dose of anti-TNF antibody, anti-IL-17 antibody or anti-TNF/anti-IL-17 DVD-Ig protein

(at day 20 after collagen II/CFA injection) one day prior to injection of one milligram of zymosan (at day 21 after collagen II/CFA injection). For another group, a therapeutic dose of anti-TNF antibody, anti-IL-17 antibody or anti-TNF/anti-IL-17 DVD-Ig protein was administered to subjects (at days 21-24 after collagen II/CFA injection) three to
5 seven days after an injection of zymosan (at day 21 after collagen II/CFA injection). Paw swelling (millimeter cubed divided by mean arthritis score; mm^3/MAS) was analyzed using calipers over a period of days.

Figure 1, panel B is a graph showing mean arthritic score (ordinate) as a function of time (abscissa) of subjects in a CIA model administered a prophylactic dose of
10 antibodies. The murine subjects were administered either: 8C11 anti-TNF antibody; MAB421 anti-IL-17 antibody; or a mixture/combination of both 8C11 anti-TNF antibody and MAB421 anti-IL-17 antibody. Control subjects were administered vehicle only.

Figure 1, panel C is a graph showing mean arthritic score (ordinate; millimeter cubed; mm^3) as a function of time (abscissa) of subjects in a CIA model administered a
15 therapeutic dose of antibodies. The murine subjects were administered either: 8C11 anti-TNF antibody; MAB421 anti-IL-17 antibody; or a mixture of both 8C11 anti-TNF antibody and MAB421 anti-IL-17 antibody. Control subjects were administered vehicle only.

Figure 1, panel D includes a set of representative images and a graph showing
20 micro CT analyzed bone volume (mm^3 ; ordinate) of tarsal bone of subjects in a CIA model administered a dose of antibodies. The subjects were administered either: 8C11 anti-TNF antibody; MAB421 anti-IL-17 antibody; or a mixture of both 8C11 anti-TNF antibody and MAB421 anti-IL-17 antibody. Control subjects were administered vehicle
25 only. Naïve subjects were not administered any dose.

Figure 1, panel E is a graph showing histological scores (ordinate) of rear paws of subjects in a CIA model administered a dose of antibodies. The subjects were administered either: 8C11 anti-TNF antibody; MAB421 anti-IL-17 antibody; or a mixture of both 8C11 anti-TNF antibody and MAB421 anti-IL-17 antibody. Control
30 subjects were administered vehicle only.

Figure 2 is a schematic representation of an anti-murine TNF/IL-17 DVD-Ig binding protein composed of 8C11 mouse anti-TNF antibody and 10F7M11 mouse anti-IL-17 antibody.

Figure 3, panels A and B, show graphs demonstrating relative gene expression of arginase 1 (panel B) and arginase 2 (panel A) in paw homogenates from naïve mice or mice with collagen-induced arthritis and treatment with either vehicle only, anti-TNF treatment only, anti-IL-17 treatment only, or a combination of both anti-TNF treatment and anti-IL-17 treatment. The data in the graphs showed decreases in both arginase 1
5 gene expression and arginase 2 gene expression in paw homogenates from mice with CIA treated with combination of anti-TNF and anti-IL-17 antibodies compared to single agent (*i.e.*, TNF treatment or IL-17 treatment) or no treatment.

Figure 4, panels A and B, show graphs demonstrating arginase 1 and 2 protein
10 levels in serum from healthy subjects, RA patients currently on methotrexate (MTX) only, or RA patients currently on methotrexate and anti-TNF treatment. The amount of arginase 2, but not arginase 1, was elevated in RA patients, regardless of treatment with MTX only or treatment with MTX and anti-TNF.

Figure 5, panels A, B, C, D, E and F, show graphs demonstrating arginine,
15 ornithine, proline, citrulline, symmetrical dimethylarginine (SDMA) and asymmetrical dimethylarginine (ADMA) levels in serum from healthy subjects, RA patients currently on methotrexate (MTX) or RA patients currently on methotrexate and anti-TNF treatment. Arginine was significantly elevated in RA patients compared to healthy controls, but no other amino acid tested showed these changes.

Figure 6, panels A, B, C and D, show graphs demonstrating gene expression of
20 arginase 2, nitric oxide synthetase 1 (NOS1), argininosuccinate lyase (ASL) and argininosuccinate synthetase 1 (ASS1) following treatment of fibroblast-like synoviocytes (FLS) with various stimuli, including recombinant TNF antibody treatment, recombinant IL-17 antibody treatment, and a combination of TNF and IL-17
25 treatments.

Figure 7, panels A and B, show graphs demonstrating gene expression of
arginase 1 and NOS 2 following treatment of FLS with various stimuli, including recombinant TNF treatment, recombinant IL-17 treatment, and a combination of TNF and IL-17 treatments.

30

DETAILED DESCRIPTION OF THE INVENTION

The present disclosure provides biomarkers associated with the urea cycle for anti-TNF and anti-IL-17 combination therapies. The present disclosure is based, at least

in part, on the observation that a combination therapy of an anti-TNF treatment and an anti-IL-17 treatment modulates (e.g., lowers or increases) the abundance of arginine as well as the level of expression of arginase II, argininosuccinate lyase, nitric oxide synthetase (NOS), 1 and argininosuccinate synthetase 1 protein or nucleic acid in a
5 subject with rheumatoid arthritis, relative to a their expression in a control subject or control subject population, indicating that the combination therapy is, or will be, effective in treating the subject for rheumatoid arthritis. Accordingly, the present invention is useful for (i) determining whether a subject will respond to a combination therapy comprising an anti-TNF treatment and an anti-IL-17 treatment; (ii) monitoring
10 the effectiveness of a combination therapy comprising an anti-TNF treatment and an anti-IL-17 treatment; (iii) selecting a subject for participation in a clinical trial for a combination therapy comprising an anti-TNF treatment and an anti-IL-17 treatment; (iv) identifying a combination therapy comprising an anti-TNF treatment and an anti-IL-17 treatment for treating a subject having rheumatoid arthritis and/or identifying candidate
15 substances that could be used to treat rheumatoid arthritis.

In certain embodiments, an increase in the abundance of arginine in a sample from a subject indicates that a subject is suffering from rheumatoid arthritis and that the symptoms of rheumatoid arthritis can be alleviated by the administration of a combination therapy of an anti-TNF treatment and an anti-IL-17 treatment. In other
20 embodiments, a decrease of arginine in a sample from a subject after administration of an anti-TNF treatment and an anti-IL-17 treatment shows an improvement in the progression of rheumatoid arthritis in a subject. In this circumstance, the dose of the anti-TNF treatment and an anti-IL-17 treatment can be held steady (*i.e.*, maintained at the current dose), or decreased. Alternatively, an increase of arginine in a sample from a
25 subject after administration of an anti-TNF treatment and an anti-IL-17 treatment shows a worsening of the progression of rheumatoid arthritis in a subject. In this circumstance, the dose of the anti-TNF treatment and an anti-IL-17 treatment can be increased.

In certain embodiments, the sample from the subject includes joint tissue or serum. More specifically, the sample from the subject can include synovium tissue.
30 More specifically, the sample from the subject can include FLS. FLS are a cell type located inside joints in the synovium. The synovium is a thin layer located between the joint capsule and the joint cavity that reduces friction between the joint cartilages during movement.

In certain embodiments, abundance or expression of arginine and arginase II gene products (e.g., protein or nucleic acid) can be measured in joint tissue, synovium tissue, FLS or serum. In certain embodiments, abundance or expression of argininosuccinate lyase, NOS 1 and argininosuccinate synthetase 1 gene products can be measured in joint tissue, synovium tissue, synovial fluid or FLS.

While a combination therapy of an anti-TNF treatment and an anti-IL-17 treatment leads to a decrease in the amount of arginine and arginase II in the serum of patients with rheumatoid arthritis, this decrease was not found in the serum of patients treated with an anti-TNF treatment without an anti-IL-17 treatment.

Further, the expression of a number of genes that are involved in the urea cycle is modulated in FLS when anti-TNF treatment and anti-IL-17 treatment are administered together in vivo as opposed to each administered separately. These genes include arginase II, argininosuccinate lyase, NOS 1 and argininosuccinate synthetase 1. The levels of arginase II, argininosuccinate lyase, and NOS 1 were higher in FLS administered a combination of anti-TNF and anti-IL-17 treatments, and not modulated when each treatment was administered separately. The levels of argininosuccinate synthetase 1 were decreased in FLS administered a combination of anti-TNF and anti-IL-17 treatments, and not modulated when each was administered separately.

Thus, by measuring the abundance of arginine or the mRNA or protein of arginase II, argininosuccinate lyase, NOS 1 and argininosuccinate synthetase 1, the presence and/or progression of rheumatoid arthritis can be monitored and the propriety and effectiveness of a combination therapy of an anti-TNF treatment and an anti-IL-17 treatment can be measured in a subject.

Definitions

Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. The meaning and scope of the terms should be clear, however, in the event of any latent ambiguity, definitions provided herein take precedent over any dictionary or extrinsic definition. Further, unless otherwise required by context, singular terms, e.g., those characterized by “a” or “an”, shall include pluralities, e.g., one or more biomarkers; “some”, “certain”, and “various”. In this application, the use of “or” means “and/or”, unless stated or differentiated

otherwise. Furthermore, the use of the terms “including” and “comprising,” as well as other forms of the terms, such as “includes”, “included”, “comprises”, and “comprised of”, are not limiting. Also, terms such as “element” or “component” encompass both elements and components comprising one unit and elements and components that
5 comprise more than one unit unless specifically stated otherwise.

The phrase “determining whether a subject having an inflammatory disease will respond to treatment with a combination therapy comprising an anti-TNF treatment and an anti-IL-17 treatment” means assessing the likelihood that treatment of a subject with a dose of the combination therapy will be therapeutically effective (*e.g.*, provide a
10 therapeutic benefit) or will not be therapeutically effective in the subject. Assessment of the likelihood that treatment will or will not be therapeutically effective typically can be performed before treatment has begun or before treatment is resumed. Alternatively or in combination, assessment of the likelihood of effective treatment can be performed during treatment, *e.g.*, to determine whether treatment should be continued or
15 discontinued.

The term “anti-TNF treatment” means any treatment for a TNF associated disease and/or any treatment that affects (*e.g.*, inhibits) the TNF pathway. This term includes TNF antagonists that have the effect of binding to or neutralizing, inhibiting, reducing, or negatively modulating the activity of TNF. In an embodiment, the anti-
20 TNF treatment comprises an anti-TNF binding protein. In an embodiment, the anti-TNF treatment can comprise an anti-TNF antibody, or an antigen binding fragment thereof. In an embodiment, the anti-TNF antibody is a murine antibody, a human antibody, a humanized antibody, a bispecific antibody, a chimeric antibody, a Fab, a Fab’, a F(ab’)₂, an ScFv, an SMIP, an affibody, an avimer, a versabody, a nanobody, a domain antibody,
25 or an antigen binding fragment of any of the foregoing.

In an embodiment, the anti-TNF antibody comprises a human anti-TNF- α antibody, *e.g.*, Adalimumab, or an antigen binding fragment thereof (see U.S. Patent No. 6,090,382). In another embodiment, the anti-TNF antibody comprises a humanized anti-TNF antibody, *e.g.*, infliximab, or an antigen binding fragment thereof. In another
30 embodiment, the anti-TNF binding protein comprises a fusion protein, *e.g.*, etanercept, or an antigen binding fragment thereof.

In other embodiments, the anti-TNF treatment comprises methotrexate, an analog thereof, or a pharmaceutically acceptable salt thereof.

In an embodiment, the anti-TNF treatment comprises a multispecific binding protein. In an embodiment, the multispecific binding protein comprises a DVD-binding protein such as, for example, a DVD-Ig molecule, a hDVD-Ig molecule, a tDVD-Ig molecule, a rDVD-Ig molecule, a pDVD-Ig molecule, a mDVD-Ig molecule, a coDVD-Ig molecule, a bbbDVD-Ig molecule, a clDVD-Ig molecule, or a rcDVD-Ig molecule.

The term "anti-IL-17 treatment" means any treatment for an interleukin 17 (IL-17)-associated disease and/or any treatment that affects (*e.g.*, inhibits) the IL-17 pathway. This term includes IL-17 antagonists that have the effect of binding to or neutralizing, inhibiting, reducing, or negatively modulating the activity of IL-17. In an embodiment, the anti-IL-17 treatment comprises an anti-IL-17 binding protein. In an embodiment, the anti-IL-17 treatment comprises a fusion protein. In an embodiment, the anti-IL-17 treatment comprises an anti-IL-17 antibody, or an antigen binding fragment thereof. In an embodiment, the anti-IL-17 antibody comprises a human antibody, *e.g.*, secukinumab and RG7624, or an antigen binding fragment thereof. In an embodiment, the anti-IL-17 antibody comprises a humanized antibody, for example ixekizumab, 10F7, B6-17, or an antigen binding fragment thereof.

In an embodiment, the anti-IL-17 treatment comprises methotrexate, an analog thereof, or a pharmaceutically acceptable salt thereof. In an embodiment, the anti-IL-17 treatment can include a multispecific binding protein.

In certain embodiments, dual anti-TNF/anti-IL-17 treatments can be provided in one molecule. In certain embodiments, these molecules are ABT-122 (D2E7-GS10-B6-17; see Table 1) or ABBV-257 (HMAK199-1-GS10-H10F7-M11; Table 2).

Table 1: Amino Acid Sequence of ABT-122, an Anti-TNF/IL-17 DVD-Ig Binding Protein

DVD HEAVY VARIABLE D2E7-GS10-B6-17 DVD-Ig Protein	SEQ ID NO.:21	EVQLVESGGGLVQPGRSLRLSCAASGFTFD DYAMHWVRQAPGKGLEWVSAITWNSGHIDY ADSVEGRFTISRDNKNSLYLQMNSLRAED TAVYYCAKVSYLSTASSLDYWGQGLVTVSS SGGGGSGGGGSEVQLVQSGAEVKKPGSSVK VSCKASGGSFGGYGIGWVRQAPGQGLEWMG GITPFFGFADYAQKFQGRVTITADESTTTA YMELSGLTSDDTAVYYCARDPNEFWNGYYS THDFDSWGQGTITVTVSS
D2E7 VH*	SEQ ID NO.:22	EVQLVESGGGLVQPGRSLRLSCAASGFTFD DYAMHWVRQAPGKGLEWVSAITWNSGHIDY ADSVEGRFTISRDNKNSLYLQMNSLRAED TAVYYCAK VSYLSTASSLDY WGQGLVTVSS S

LINKER	SEQ ID NO.:23	GGGGSGGGGS
B6-17 VH*	SEQ ID NO.:24	EVQLVQSGAEVKKPGSSVKVSKASGGSGF GYGIGWVRQAPGQGLEWMGGITPFFGFADY AQKFQGRVTITADESTTTAYMELSGLTSD TAVYYCARD PNEFWNGYYSTHDFDSW GQGT TVTVSS
CH	SEQ ID NO.:25	ASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTWNSGALTSGLVHTFPAVLQSS GLYSLSSVTVPSSSLGTQTYICNVNHKPS NTKVDKKVEPKSCDKTHTCPPCPAPPELLGG PSVFLFPPKPKDTLMISRTPEVTCVVDVVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSRDE LTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFCFSVMHEALHNHYTQKSLSLSPGK
DVD LIGHT VARIABLED2E7- GS10-B6-17 DVD-Ig Protein	SEQ ID NO.:26	DIQMTQSPSSLSASVGDRVTITCRASQGIR NYLAWYQQKPGKAPKLLIYAASLTQSGVPS RFGSGSGTDFTLTISLQPEDVATYYCQR YNRAPYTFGQGTKVEIKRGGSGGGSGEIV LTQSPDFQSVTPKEKVTITCRASQDIGSEL HWYQQKPDQPPKLLIKYASHSTSGVPSRFS GSGSGTDFTLTINGLEAEADAGTYCHQTDS LPYTFGPGTKVDIKR
D2E7 VL*	SEQ ID NO.:27	DIQMTQSPSSLSASVGDRVTITCRASQGIR NYLAWYQQKPGKAPKLLIYAASLTQSGVPS RFGSGSGTDFTLTISLQPEDVATYYCQR YNRAPYTFGQGTKVEIKR
LINKER	SEQ ID NO.:28	GGSGGGGSG
B6-17 VL*	SEQ ID NO.:29	EIVLTQSPDFQSVTPKEKVTITCRASQDIG SELHWYQQKPDQPPKLLIKYASHSTSGVPS RFGSGSGTDFTLTINGLEAEADAGTYCHQ TDSLPTYTFGPGTKVDIKR
CL	SEQ ID NO.:30	TVAAPSVFIFPPSDEQLKSGTASVVCCLNN FYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYLSLSLTLSKADYEKHKVYACEVTH QGLSSPVTKSFNRGEC

*Note that the component CDRs of the VH and VL binders are in bold.

Table 2: Amino Acid Sequence of ABBV-257, an Anti-TNF/IL-17 DVD-Ig Binding Protein

DVD HEAVY VARIABLE HMAK199-1-GS10- H10F7-M11 DVD	SEQ ID NO.:11	EVQLVQSGAEVKKPGASVKV SCKASGYTFANYGIIWVRQA PGQGLEWMGWINTYTGKPTY AQKFQGRVTMTTDTSTSTAY MELSSLRSEDTAVYYCARKL FTTMDVTDNAMDYWGQGTTV TVSSGGGGSGGGGSEVQLVQ SGAEVKKPGSSVKVSKASG YTFTDYEIHWVRQAPGQGLE WMGVNDPESGGTFYNQKFDG RVTLTADESTSTAYMELSSL RSEDTAVYYCTRYSKWDSFD GMDYWGQGTITVTVSS
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HMAK199-1VH*	SEQ ID NO.:12	EVQLVQSGAEVKKPGASVKV SCKASGYTF FANYGI IWVRQA PGQGLEWMG WINTYTGKPTY AQKFQ GRVTMTTDTSTSTAY MELSSLRSEDTAVYYCARK L FTTMDVTDNAMDY WGQGTTV TVSS
LINKER	SEQ ID NO.:13	GGGGSGGGGS
H10F7-M11 VH*	SEQ ID NO.:14	EVQLVQSGAEVKKPGSSVKV SCKASGYTF TDYEIH WVRQA PGQGLEWMG VNDPESGGTFY NQKFD GRVTTLTADESTSTAY MELSSLRSEDTAVYYCT RY S KWDSFDGMDY WGQGTTVTVS S
CH CG1234, 235 MUT Z NONA	SEQ ID NO.:15	ASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSS GLYSLSSVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEP KSCDKTHTCPPCPAPEAAGG PSVFLFPPKPKDTLMISRT EVTCTVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRW QQGNVFCFSVMHEALHNHYT QKSLSLSPGK
DVD LIGHT VARIABLE HMAK199-1-GS10- H10F7-M11DVD	SEQ ID NO.:16	DIQMTQSPSSLSASVGDRV ITCRASQDISQYLNWYQQK GKAPKLLIYYTSRLQSGVPS RFGSGSGTDFTLTISLQ EDFATYFCQQGNTWPPTFGQ GTKLEIKRGGSGGGSGDIQ MTQSPSSLSASVGDRVITC RASSGIISYIDWFQQKPGKA PKRLIYATFDLASGVPSRFS GSGSGTDYTLTISLQPEDF ATYYCRQVGSYPETFGQGTK LEIKR
HMAK199-1 VL*	SEQ ID NO.:17	DIQMTQSPSSLSASVGDRV ITC RASQDISQYLNWYQQK GKAPKLLIY YTSRLQ SGVPS RFGSGSGTDFTLTISLQ EDFATYFC QQGNTWPPT FGQ GTKLEIKR
LINKER	SEQ ID NO.:18	GGSGGGGSG
H10F7-M11VL*	SEQ ID NO.:19	DIQMTQSPSSLSASVGDRV ITC RASSGIISYIDWFQQK GKAPKRLIY ATFDLAS GVPS RFGSGSGTDYTLTISLQ

		EDFATYY CRQVGSYPET FGQ GTKLEIKR
CL	SEQ ID NO.:20	TVAAPSVFIFPPSDEQLKSG TASVVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQDS KDSTYLSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKS FNRGEC

*Note that the component CDRS of the VH and VL binders are in bold.

Antibodies used in immunoassays to determine the level of expression of the biomarkers of the invention may be labeled with a detectable label. The term “labeled”,
5 with regard to the probe or antibody, encompasses direct labeling of the probe or antibody by incorporation of a label (*e.g.*, a radioactive atom), coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a
10 fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin.

In an embodiment, the antibody is labeled, *e.g.*, a radio-labeled, chromophore-labeled, fluorophore-labeled, or enzyme-labeled antibody. In other embodiments, an antibody derivative (*e.g.*, an antibody conjugated with a substrate or with the protein or
15 ligand of a protein-ligand pair, such as biotin-streptavidin), or an antibody fragment (*e.g.*, a single-chain antibody, or an isolated antibody hypervariable domain) which binds specifically with the biomarker is used.

The term “biomarker” means a substance that is used as an indicator of a biologic state, *e.g.*, amino acids, proteins, genes, DNA, cDNA, messenger RNAs
20 (mRNAs, microRNAs (miRNAs)); heterogeneous nuclear RNAs (hnRNAs), and proteins, or portions thereof.

The terms “level of expression” or “expression pattern” mean a quantitative or qualitative summary of the expression or abundance of one or more biomarkers in a subject, such as in comparison to a standard or a control.

25 The term “baseline abundance” means the level of biomarker present in a sample as a comparator to a subject or a sample that has been treated with an anti-TNF and anti-IL-17 treatment. In an embodiment, the baseline abundance refers to the level of biomarker in a normal individual or population of individuals. In an embodiment, the baseline abundance refers to the level of biomarkers in a subject with rheumatoid

arthritis prior to treatment with the anti-TNF and anti-IL-17 treatment. In an embodiment, the baseline abundance refers to the level of biomarkers in a healthy tissue from a subject with rheumatoid arthritis. In an embodiment, the baseline abundance refers to the level of biomarkers in a healthy tissue from a subject that was collected
5 from the subject during a period in which the subject was not experiencing symptoms of rheumatoid arthritis. Thus, regardless of the “baseline abundance” measurement chosen, the biomarkers of the invention that are increased in subject samples following anti-TNF and anti-IL-17 treatment and the biomarkers of the invention that are decreased in subjects following anti-TNF and anti-IL-17 treatment can be used across disease
10 indications to determine responsiveness to the anti-TNF and anti-IL-17 treatment.

The terms “higher level of expression” and “increase in the level of expression” mean an expression level in a test sample that is greater than the standard error of the assay employed to assess expression, and is at least 50% greater, or two, three, four, five, six, seven, eight, nine, or ten or more times the expression level in a control sample
15 (*e.g.*, a sample from a healthy subject not afflicted with RA, and/or a sample from a subject(s) having slow disease progression and/or, the average expression level of one or more of the biomarkers disclosed herein in several control samples).

The terms “lower level of expression” and “decrease in the level of expression” mean an expression level in a test sample that is less than the standard error of the assay employed to assess expression, and at least 50% greater, or two, three, four, five, six,
20 seven, eight, nine, or ten or more times less than the expression level in a control sample (*e.g.*, a sample from a subject with rapid disease progression and/or a sample from the subject prior to administration of a portion of a therapy for RA, and/or the average expression level one or more of the biomarkers disclosed herein in several control
25 samples).

The term “component of the urea cycle” means an enzyme, cofactor, substrate, product, byproduct, metabolite, precursor or other component associated with the urea cycle and its related pathways. The urea cycle (also known as the ornithine cycle) is a cycle of biochemical reactions occurring in many animals that metabolizes ammonia to
30 produce urea. Exemplary components of the urea cycle include ornithine, carbamoyl phosphate, citrulline, argininosuccinate, fumarate, arginine, urea, aspartate, carbamoyl phosphate synthetase I (CPS1), ornithine transcarbamoylase (OTC), arginosuccinate synthetase or arginosuccinate synthase (ASS), arginosuccinate lyase or arginosuccinase

(ASL), arginases such as Arginase 1 (ARG1) and Arginase II (ARG2), and nitric oxide synthetases such as nitric oxide synthetase (NOS) 1.

Arginase proteins are polypeptides that catalyze the hydrolysis of arginine to form urea and ornithine. See U.S. Patent No. 8,679,479.

5 The term “arginase I” means a protein that is highly expressed in the cytosol of hepatocytes, and functions in nitrogen removal as the final step of the urea cycle. See U.S. Patent No. 9,109,218. Arginase sequences (including human arginase I gene sequences) are disclosed in Haraguchi *et al.* (1987) Proc. Natl. Acad. Sci. USA 84; 412-415 and U.S. Patent Nos. 7,741,310 and 9,050,340.

10 The term “arginase II” means a protein that catalyzes the hydrolysis of arginine to ornithine and urea. In certain embodiments, the amino acid and mRNA sequences for the human arginase II protein (ARG2) and mRNA are provided below.

Table 3: Human Arginase II Amino Acid Sequence

SEQ ID NO	123456789012345678901234567890123456789012345678901234567890
91	MSLRGSLSRLLQTRVHSILKKS VHSVAVIGAPFSQGQKRKGVEHGPA AIREAGLMKRLSS LGCHLKDFGDLSTFPVKDDLYNNLIVNPRSVGLANQELA EVVSRAVSDGYSCVTLGGDH SLAIGTISGHARHCPDLCVWVDAHADINTPLTSSGNLHGQPVSFLLRELQDKVPQLPG FSWIKPCISSASIVYIGLRDVPPEHFILKNYDIQYFSMRDIDRLGIQKVMERTFDLLIG KRQRPIHLSFDIDAFDPTLAPATGTPVVGGLTYREGMYIAEEIHNTGLLSALDLVEVNPQ LATSEEEAKTTANLAVDVIASSFGQTREGGHIVYDQLPTPSSPDESENQARVRI

Table 4: Human Arginase II Nucleotide Sequence

SEQ ID NO	123456789012345678901234567890123456789012345678901234567890
92	cacggggcgggcgggacgctggcgcgggtaggtaagagcagcgggcgggcggtggcgctcac tccccggttccaaccgcgcggagcctctgccttggagattctcagtgctgcggatcatgt ccctaaggggcagcctctcgcgtctcctccagacgcgagtgcatcctcctgaagaaat ccgtccactccgtggctgtgataggagccccgttctcacaagggcagaaaagaaaggag tggagcatggtcccgcgtgccataagagaagctggcttgatgaaaaggctctccagtttgg gctgccacctaaaagactttggagatttgagttttactccagtccccaagatgatctct acaacaacctgatagtgaatccacgctcagtggggtcttgccaaccagggaactggctgagg tggttagcagagctgtgtcagatggctacagctgtgtcactgggaggagaccacagcc tggcaatcgggtaccattagtgccatgcccagactgcccagacctttgtgtgtctggg ttgatgcccatgctgacatcaacacaccccttaccacttcacaggaaatctccatggac agccagtttcatttctcctcagagaactacaggataaggtaccacaactcccaggatttt cctggatcaaaccttgatctcttctgcaagtattgtgtatattggtctgagagacgtgg accctcctgaacattttattttaagaactatgatatccagtattttccatgagagata ttgatcgacttggtatccagaaggtcatggaacgaacatttgatctgctgattggcaaga gacaaagaccaatccatttgagtttgatattgatgcatttgacctacactggctccag ccacagggaactcctgttgctgggggactaacctatcgagaaggcatgtatattgctgagg aaatacacataacagggttgctatcagcactggatctgttggaagtcaatcctcagttgg

	1ccacctcagaggaagaggcgaagactacagctaacctggcagtagatgtgattgcttca agctttggtcagacaagagaaggagggcatattgtctatgaccaacttcctactcccagt tcaccagatgaatcagaaaatcaagcacgtgtgagaatttaggagacactgtgcactgac atgtttcacaacaggcattccagaattatgaggcattgagggatagatgataactaaatg gttgctcgggtcaatactgccttaatgagaacatttacacattctcacaattgtaaagt tcccctctattttggtgaccaatactactgtaaatgtatttggtttttgcagttcacag ggtattaatatgctacagtactatgtaaatttaaagaagtcataaacagcatttattacc ttggtatatcatactggctcttgttgctgttggttccttcacatttaagtggttttcatct ttctccctcctccacagcctggctatacagtgcatccttgaaactgtcagcccacagca gcaatatgcttattctatccacatccctaacatcatgcattcacaaaggtaaaagtctgg tcacaaaacccttcctatagaagttcaatggctgcgaaagaattttagtaaaaccaggc ctccaggatggcgagctccagtaagatgataatggaaagcagcagcttggtggtgtca ctctacaaagagaagcaaagtggggagtagtcagaagtttggataaccttccttctaacc atgttgggttagacctgggaccacggctggatactctgaggctgtatgttgatcacac agccacttagcaggaagtactcataaggttcttttagctgtcacttagggataacactgtc tacctcacagaaatgttaactgagacaataaaaaccaaagcataaaaatggattctgaa a
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- The terms “argininosuccinate lyase” and “arginosuccinase” mean a protein that catalyzes the reversible breakdown of argininosuccinate producing the amino acid arginine and fumarate. The amino acid and mRNA sequences for the human
- 5 argininosuccinate lyase protein and mRNA are provided below.

Table 5: Human Argininosuccinate Lyase Amino Acid Sequence

SEQ ID NO	123456789012345678901234567890123456789012345678901234567890
93	masesgklwgrfvgavdpimekfnsaiaydrhlwevdvqgskaysrglekaglltkaem dqilhglldkvaewaaggtfklnsndedihtanerrlkeligatagklhtgrsrndqvvt lrlwmrqtctslsgllwelirtmvdraeaerdvlfpgythlqraqpirshwilshaval trdserllevrkrinvlplgsgaiagnplgvdrellraelnfgaitlnsmatserdfva eflfwrslcmthlsrmaedlilyctkefsfvqlsdaystgsslmppqknpdslelirska grvfgrcagllmtlkglpstynkdlqedkeavfevsdtmsavqlvatgvistlqihqenm ggalspdmldatlayylvrkgmpfrqaheasgkavfmaetkgvalnqlslqelqtisplf sgdvicvwdyghsveqygalggtarssvdwqirqvrrallqaqqa

Table 6: Human Argininosuccinate Lyase Nucleotide Sequence

SEQ ID NO	123456789012345678901234567890123456789012345678901234567890
94	gagacccggaggaccgaagcttccggacgacgaggaaccgcccacatggcctcgagag tggaagctttgggtggccggtttgtgggtgcagtggaacccatcatggagaagttcaa cgcgtccattgcctacgaccggcacctttgggaggtggatgttcaaggcagcaaagccta cagcaggggctggagaaggcagggtcctcaccaaggccgagatggaccagatactcca tgacctagacaaggtggctgaggagtgggccagggcaccttcaaactgaactccaatga taggacatccacacagccaatgagcgccgctgaaggagctcattggtgcaacggcagg gaagctgcacacgggacggagccgaatgaccaggtggtcacagacctcaggctgtggat gcggcagacctgctccacgctctcgggcctcctctgggagctcattaggaccatggtgga tcgggcagaggcggaacgtgatgttctcttccggggtacacccatttgcagagggccca

	gccccatccgctggagccactggattctgagccacgccgtggcactgacccgagactctga gcggtctgctggaggtgcggaagcggatcaatgtcctgccccctggggagtgggggcattgc aggcaatccccctgggtgtggaccgagagctgctccgagcagaactcaactttggggccat cactctcaacagcatggatgccactagttagcgggactttgtggccgagttcctgttctg ggcttcgctgtgcatgacccatctcagcaggatggccgaggacctcatcctctactgcac caaggaattcagcttcgtgcagctctcagatgcctacagcacgggaagcagcctgatgcc ccagaagaaaaaccccgacagtttgagctgatccggagcaaggctgggctgtgtttgg gcggtgtgcgggctcctgatgacctcaagggaactcccagacctaacaagaagactt acaggaggacaaggaagctgtgtttgaagtgtcagacactatgagtgccgtgctccagggt ggccactggcgctcatctctacgctgcagattcaccaagagaacatgggacaggctctcag ccccgacatgctggccactgaccttgctattacctggctccgcaaagggtgacattccg ccaggccacgaggcctccgggaaagctgtgttcattggccgagaccaaggggtgcacct caaccagctgtcactgcaggagctgcagaccatcagccccctgttctcgggcgacgtgat ctgcgtgtgggactacgggcacagtgtggagcagtatgggtgcctggggcgactgcgcg ctccagcgtcgactggcagatccgccagggtgctggcgctactgcaggcacagcaggccta ggtcctccacacctgccccctaataaagtgggctgcgagaggaaaaaaaaaaaaaaaaaa aaaaaaaaaa
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The term “nitric oxide synthase 1” means a protein that synthesizes nitric oxide (NO) from arginine. NO is a messenger molecule with diverse functions throughout the body. The amino acid sequence for the human nitric oxide synthase 1 protein is provided below. The nucleic acid sequence for human nitric oxide synthase 1 protein is provided under NCBI Reference Sequence: NM_001204218.1, which is incorporated herein by reference in its entirety.

Table 7: Human Nitric Oxide Synthase 1 Amino Acid Sequence

SEQ ID	123456789012345678901234567890123456789012345678901234567890
NO	
95	MEDHMFVQVQIQPNVISVRLFKRKVGGGLGFLVKERVSKPPVVISDILIRGGAAEQSGLIQQA GDIILAVNGRPLVDLSYDSALEVLRGIASETHVVLILRGPEGFTTHLETTFTGDGTPKTI RVTQPLGPPTKAVDLSHQPPAGKEQPLAVDGASGPGNGPQHAYDDGQEAAGSLPHANGLAP RPPGQDPAKKATRVSLQGRGENNELLEKEIEPVLSLLTSGSRGVKGGAPAKAEMKDMGIQV DRDLDGKSHKPLPLGVENDRVFNDLWCKGNVPVVLNNPYSEKEQPPTSGKQSPKNGSPS KCPREFLKVKNWETEVVLTDTLHLKSTLETGCTEYICMGSIMHPSQHARRPEDVRTKGQLF PLAKEFIDQYYSSIKRFGSKAHERLEEVNKEIDTTSTYQLKDTELIYGAKHAWRNASRC VGRIQWSKLQVFDARDCTTAHGMFNYICNHVKYATNKGNLRSAITIFPQRTDGKHDVFW NSQLIRYAGYKQPDGSTLGDPAVQFTEICIQGWKPPRGREFDVLPLLLQANGNDPELFQ IPPELVLEVPIRHPKFWEFKDLGLKWYGLPAVSNMELLEIGGLEFSACPFSGWYMGTEIGV RDYCDNSRYNILEEVAKKMNDMRKTSSLWKDQALVEINIAVLYSFQSDKVTIVDHSAT ESFIKHMEYRCRGCPADWVWVPPMSGISITPVFHQEMLNRYLTPSFYQPDPPWNTHV WKGTTNGTPTKRRALGFKKLAEAVKFSAKLMQAMAKRVKATILYATETGKSQAYAKTLCE IFKHAFDAKVMSMEEDIVHLEHETLVLVVTSTFGNGDPPENGEKFGCALMEMRHPNSVQ EERKSYKVRENSVSSYSDSQKSSGDPDLRDNFESAGPLANVRFVFGLSRAYPHFCFAF GHAVDTLLEELGGERILKMREGDELCCQEEAFRTWAKKVFKAACDVFCVGDVNIKANN SLISNDRSWKRNFRLTFVAEAPELTQGLSNVHKKRVSAARLLSPQNLQSPKSSRSTIFV RLHTNGSQELQYQPGDHLGVFPNGHEDLVNALIERLEDAPPVNMVVKVELLEERNALGV ISNWTDELRLPPCTIFQAFKYLDITTPPTPLQLQQFASLATSEKEKQRLVLVLSKGLQEY EEWKWGNPTIVEVLEEFPSIQMPATLLLTQLSLLQPRYYSISSSPDMPDEVHLTVAIV SYTRDGEPIHHGVCSSWLNRIQADELVPCFVRGAPSFHLPRNPQVPCILVPGPTGIAP FRSFWQQRQFDIQHKGMNPCPMVLVFGCRQSKIDHIYREETLQAKNKGVFRELYTAYSRE

PDKPKKYVDILQEQLAESVYRALKEQGGHIYVCGDVTMAADV LKAIQRIMTQQGKLSAE DAGVFISMRDDNRYHEDI FGVTLRTYEVTNRLRSESIAFI EESK KDTDEVFSS
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Table 8: Human Nitric Oxide Synthase 1 Nucleic Acid Sequence

SEQ ID NO	123456789012345678901234567890123456789012345678901234567890
98	ataaaagatgtatgcttttgagcccagagcggctcttttaatgaggggttgcgacgtctcc ctccccacaccataaaccagtcgggttgagcgtcactgctaattcgtttcagtgtatgat aggataaaggaggacattaagaaataaattccccctcacgacctcgctgagctcacgg ctgagtcacctacatatattatgcccgtttccagccgctgggtgaggagctacttagcgcc gcggctcctccgagggggcgccgggcagcgagcagcgccgagcggaagggtcatgatg cctcagatctgatccgcacttaacaggctggcaatgaagatacccagagaatagttcaca tctatcatgcggtcacttctagacacagccatcagacgcatctcctcccccttctgctga ccttagggacacgtcccaccgctctcttgacgtctgcctggtcaaccatcacttcctta gagaataaggagagagggcggtgcaggaaatcatgccaccgacgggcccaccagccatgag tgggtgacgctgagctgacgtcaaagacagagaggggtgaagccttgtagcacctgtca ccccgctcctgctctccgtgtagcctgaagcctggatcctcctggtgaaatcatcttgg cctgatagcattgtgaggtcttcagacaggacctcgggaagctagtaccatggaggat cacatgttcggtgttcagcaaatccagcccattgtcatttctgttcgtctcttcaagcgc aaagtgggggctgggatttctggtgaaggagcgggtcagtaagccgcccgtgatcatc tctgacctgattcgtggggcgccgcagagcagagtgccctcatccaggccggagacatc attcttgcggtcaacggccgccccttggtggacctgagctatgacagcgccctggaggta ctgagaggcattgcctctgagacccaagtggtcctcattctgagggggcctgaaggttc accacgcacctggagaccacctttacaggtgatgggaccccccaagaccatccgggtgaca cagcccctgggtccccccaccaaagcgtggatctgtcccaccagccaccggccggcaaa gaaagccccctggcagtggtggggtcctcggttcccggaatgggctcagcatgcctac gatgatgggcaggaggctgggtcactcccccatgccaacggcctggccccagggcccca ggccaggacccccggaagaaagcaaccagagtcagcctccaaggcagaggggagaacaat gaactgctcaaggagatagagcctgtgctgagccttctcaccagtgaggagcagaggggtc aaggagggggcacctgccaaggcagagatgaaagatatgggaatccagggtggacagagat ttggacggcaagtcacacaaaacctctgcccctcggcgtggagaacgacggagcttcaat gacctatgggggaagggaatgtgcctgtgcgtcctcaacaaccatattcagagaaggag cagccccccacctcaggaaaacagtccccccacaaagaatggcagccccctccaagtgtcca cgcttctcaagggtcaagaactgggagactgaggtggttctcactgacacctccacctt aagagcacattggaaacgggatgactgagtacatctgcatgggtccatcatgcatcct tctcagcatgcaaggaggcctgaagacgtccgcacaaaaggacagctcttccctctcgcc aaagagtttattgatcaatactattcatcaattaaaagatttgggtccaaagcccacatg gaaaggctggaagaggtgaacaaagagatcgacaccactagcacttaccagctcaaggac acagagctcatctatggggccaagcagcctggcggaatgcctcgcgctgtgtgggcagag atccagtggttccaagctgcaggtattcgatgccctgactgcacacggccacgggatg ttcaactacatctgtaacctgtcaagtatgccaccaacaaagggaacctcaggtctgcc atcaccatattccccagaggacagacggcaagcacgacttccgagctctggaactccag ctcatccgtacgctggctacaagcagcctgacgggtccacctgggggacccagccaat gtgagttcacagagatatgcatacagcagggtggaaaccgctagaggccgcttcgat gtctgcccgtcctgcttcaggccaacggcaatgacctgagctcttccagattcctcca gagctggtgttggaagttcccatcaggcaccccccaagtttgagtggttcaaggacctgggg ctgaagtggtagcgctcccccgctgtccaacatgctcctagagattggcgccctggag ttcagcgctgtccttccagtggtggtacatgggcacagagattggtgtccgcgactac tgtgacaactccgctacaatatcctggaggaagtggccaagaagatgaacttagacatg aggaagacgtcctcctgtggaaggaccaggcgctgggtggagatcaatatcgcggttctc tatagcttccagagtgacaaagtgaccttgttgacctcactccgccaccgagtccttc attaagcacatggagaatgagtaccgctgcccgggggggctgcctgccgactgggtgtgg atcgtgccccccatgtccggaagcatccccctgtgttccaccagagatgctcaactac cggctcacccccctccttgaataccagcctgatccctggaacacgcatgtctggaaggc accaacgggacccccacaaagcggcgagccattggcttcaagaagctagcagaagctgtc aagttctcgcccaagctgatggggcaggctatggccaagagggtgaaagcgaccatcctc tatgccacagagacaggcaaatcgcaagcttatgccaaagacctgtgtgagatcttcaaa cacgcctttgatgccaagggtgatgtccatggaagaatatgacattgtgcacctggaacat

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	aaaaattaaaatgaaagaccaggtgctgggattaaggaacacaggtctgagggctgag ggaaggggcctgcctcccagggagtgcaacatagatgttccccatgaacagggatttgact ttggaggccaacctggcctggcctctgccctttatctcacactccctatccttggccac tgccagtcctgccttggtggcaagggggcccaaaaagaaaagctgcccttccccaaatgt aaggacccaggtacactttcacccgtggaaagcagtgctgtcgcagagtgctgtttcctat taatacttatcaaagccatgtgcgagggaggtggtcagctgtcaatatgccttagtatgt ttatatgagtttgttttgttctaaaatacccaaacagttctggtaacgggggctatgcc cgtctggcccaaacacagtcctgttattaacgagatggcctggcagggcggaacaaatc tgctccatgcactgcttcctgtagtcttttagaaagtaactccaggacatcgaaagtgc cagatttgactcctaagttctaggagactgtagcgcagggctgtgtcaaccttagcactat tggcatttggggctgggtaattctttctgtgggggcccgtcttgggtactgtaggaagct gagcagcattcctggcctccatccacaagatacctgtagcagtgctgtgccaacggtaac aatcaagtatgtcatcagacattgccaatgtccccagggggcaacacccctctcttggga cttcaggggtcaagagaatctctgtggctaccccaggacttctcattatagatttccctgg agcacgcagcagaaactttgcctagcccagtggttgtttccattatctgctgccaaagt ggatttgaggggtgtccgggggagggggcatggggagggcagtatgctttcaaaaaccct ccaggccaggcggtgggtgctcatgctgtaatcacaggactttgggagggcaggctgg cagatcacttgaggctgggagttagagacaaacctggctaaccatggcaaacctcgtctc tactaaaaatacaaaaatcagcccggtgggtggcgggcatctgtaatcccatctactcg ggaggctgagggcaggagaattacttgaaacccaggaggcagaggctgcagtgcagccgagat ggcaccactgcactccagctgttgacagaatgagaccctgtggaaaaaaaaaaaaaagc cctcccatgccagaacagaggatggcagtgctgtttcaataagacactgtgtccttgggtgt tggttctgattaagactcactgagatccagtgctcttgagctgggtctcagtcacctccc atgtcctgtgctctgccgccactgttttcattgttgtgttctcgttgtgattgttaagac tcacactcctggctcagcagtggttttccagaaggcccaagagcgggtgccggggcacccc acgtcgagtggtccgttccgggcttgggaagctggggaggtgggcagacctggtcgcatc tcaccacacacacacacacacacacacacacacacacgctgtcagaaactcggcgctcccc ctacctctgagctctcaatgctgctaattctctgccaagtgtccctgtgctccagcacctt ccttgaaggactgacgccacccccacgctctttgaggggtgtccaggtgtgtttgtcg catgctcttctctgtatagttctcatcttccaattttatgggattcaacaaaagcctat tatgcttgtttgcattatgggtacaataattaaaaagtgattcaaaaaaa
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The term “argininosuccinate synthetase 1” means a protein that catalyzes the synthesis of argininosuccinate from citrulline and aspartate and forms the third step of the urea cycle. The amino acid and mRNA sequences for the human argininosuccinate synthetase 1 protein and mRNA are provided below.

Table 9: Human Argininosuccinate Synthetase 1 Amino Acid Sequence

SEQ ID NO	123456789012345678901234567890123456789012345678901234567890
96	MSSKGSVVLAYSGGLDTSCI LVWLKEQGYDVIAYLANIGQKEDFEEARKKALKLGAKKVF IEDVSREFVEEFIWPAIQSSALYEDRYLLGTSLARPCIARKQVEIAQREGAKYVSHGATG KGNDQVRFEFELSCYSLAPQIKVIAFWRMPEFYNRFKGRNDLMEYAKQHGIPIPVTPKNPWS MDENLMHISYEAGILENPKNQAPPGLYTKTQDPAKAPNTPDILLEIEFKKGVPVKVTNVKD GTHQTSLELFMYLNEVAGKHGVGRIDIVENRFIGMKS RGIYETPAGTILYHAHLDIEAF TMDREVRKIKQGLGLKFAELVYTGFWHSPCEFFVRHCAIKSQERVEGKVQVSVLKGQVYI LGRESPLSLYNEELVSMNVQGDYEPTDATGFININSLRLKEYHRLQSKVTAK

Table 10: Human Argininosuccinate Synthetase 1 Nucleotide Sequence

SEQ ID NO	12345678901234567890123456789012345678901234567890
97	gcccggcgccccctgggaggggtgagccggcgccggggcccaggccccggacctggtgggagg cggggggaggtggggacgaggcctggggagggcgggcccccgccatctgcagggtggctgtg aacgctgagcgggtccaggcgggggccggggccccggggcggggtctgtggcgcgctccc cgccacgtgtccccgggtcacccggccctgcccccgggccctgtgcttataacctgggatgg gcacccctgccagtcctgtctgcccgcctgccaccgctgcccgagccccgagtggttact gcactgtgaaaacagattccagacgcgggaactcacgcctccaatcccagacgctatgt ccagcaaaggctccgtggttctggcctacagtgggcgccctggacacctcgtgcacccctcg tgtggctgaaggaacaaggctatgacgtcattgcctatctggccaacattggccagaagg aagacttcgaggaagccagggaagaaggcactgaagcttggggccaaaaagggtgttcattg aggatgtcagcagggagtttggaggagttcatctggccggccatccagtcacgcgcac tgtatgaggaccgctacctcctgggcacctctcttgccaggccctgcatcgcccgcaaac aagtggaaatcgcccagcgggagggggccaagtatgtgtccacggcgccaaggaaagg ggaacgatcagggtccggtttgagctcagctgctactcactggccccccagataaagggtca ttgctccctggaggatgcctgaattctacaaccgggttcaaggggccgaatgacctgatgg agtacgcaaagcaacacgggattcccatcccggtcactcccaagaaccggtggagcatgg atgagaacctcatgcacatcagctacgagggtggaatcctggagaaccccaagaaccaag cgctccagggtctctacacgaagaccaggaccagccaaagcccccaacacccctgaca ttctcgagatcgagttcaaaaaaggggtccctgtgaaggtagaccaacgtcaaggatggca ccaccaccagacctccttgagctcttcatgtacctgaacgaagtgcggggcaagcatg gcgtgggcccgtattgacatcgtggagaaccgcttcattggaatgaagtcccaggtatct acgagaccccgagcaggcaccatcctttaccatgctcatttagacatcgaggcccttcacca tggaaccgggaagtgcgcaaaatcaacaaggcctgggcttgaaatttgctgagctggtgt ataccggtttctggcacagccctgagtggtgaatttgtccgccactgcatcgccaagtcctc aggagcgagtggaagggaagtgcagggtgtccgtcctcaaggggcagggtgtacatcctcg gccgggagtcctccactgtctctctacaatgaggagctggtgagcatgaacgtgcagggtg attatgagccaactgatgccaccgggttcacatcaacatcaattccctcagggtgaaggaat atcatcgtctccagagcaaggctcactgccaatagaccggtgtacaatgaggagctgggg cctcctcaatttgagatcccccaagtacaggcgctaattgtgtgataatttgtaattg tgacttgctctccccggctggcagcgtagtggggctgccaggccccagctttgtccctg gtccccctgaagcctgcaaacgttgtcatcgaagggaagggtggggggcagctgcggtgg ggagctataaaaaatgacaattaaaagagacactagtcttttatttctaaaaaaaaaaaaa aaa

Reference to a gene encompasses naturally occurring or endogenous versions of the gene, including wild type, polymorphic or allelic variants or mutants (*e.g.*, germline mutation, somatic mutation) of the gene, which can be found in a subject. In an embodiment, the sequence of the biomarker gene is at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to a biomarker described herein, *e.g.*, of arginase II, argininosuccinate lyase, NOS 1 and argininosuccinate synthetase 1 protein or nucleic acid, or a homolog, portion or derivative thereof. Sequence identity can be

determined, *e.g.*, by comparing sequences using NCBI BLAST (*e.g.*, Megablast with default parameters).

In an embodiment, the level of expression of the biomarker is determined relative to a control, a control level or second sample, such as the level of expression of the biomarker in normal tissue (*e.g.*, a range determined from the levels of expression of the biomarker observed in normal tissue samples). In an embodiment, the level of expression of the biomarker is determined relative to a control sample, such as the level of expression of the biomarker in samples from other subjects suffering from rheumatoid arthritis or free of rheumatoid arthritis. For example, the level of expression of the biomarker in samples from other subjects can be determined to define levels of expression that correlate with sensitivity to treatment with an anti-TNF treatment and/or an anti-IL-17 treatment, and the level of expression of the biomarker in the sample from the subject of interest is compared to these levels of expression.

The term “binding moiety” means the portion of a substance that specifically binds to a given molecule. In certain embodiments, binding moieties used according to the methods disclosed herein specifically bind for example to arginase II, argininosuccinate lyase, NOS 1 and argininosuccinate synthetase 1 protein or nucleic acid, or a homolog, portion or derivative thereof.

The terms “known standard level,” “control level” and “baseline abundance” mean an accepted or pre-determined expression level of the biomarker, for example arginase II, argininosuccinate lyase, NOS 1 and argininosuccinate synthetase 1 protein or nucleic acid, or a homolog, portion or derivative thereof, which is used to compare the expression level of the biomarker in a sample derived from a subject. In one embodiment, the baseline abundance of the biomarker is the average expression level of the biomarker in samples derived from a population of subjects, *e.g.*, the average expression level of the biomarker in a population of subjects with or without rheumatoid arthritis. In another embodiment, the baseline abundance of the biomarker is the average expression level of the biomarker in samples derived from a population of subjects, *e.g.*, the average expression level of the biomarker in a population of subjects without rheumatoid arthritis. In another embodiment, the baseline abundance constitutes a range of expression of the biomarker in normal tissue. In another embodiment, baseline abundance refers to a pre-treatment level of the biomarker in a subject.

Control levels of expression of biomarkers of the invention may be available from publicly available databases. In addition, Universal Reference Total RNA (Clontech Laboratories) and Universal Human Reference RNA (Stratagene) and the like can be used as controls. For example, qPCR can be used to determine the level of
5 expression of a biomarker, and an increase in the number of cycles needed to detect expression of a biomarker in a sample from a subject, relative to the number of cycles needed for detection using such a control, is indicative of a low level of expression of the biomarker.

The terms "antagonist" and "inhibitor" mean a modulator that, when contacted
10 with a molecule of interest causes a decrease in the magnitude of a certain activity or function of the molecule compared to the magnitude of the activity or function observed in the absence of the antagonist. Particular antagonists of interest include those that block or modulate the biological or immunological activity of human TNF- α and/or IL-17. Antagonists and inhibitors of human TNF- α and IL-17 may include, but are not
15 limited to, proteins, nucleic acids, carbohydrates, or any other molecules, which bind to human TNF- α and IL-17, and/or modulate expression or activity of human TNF- α and IL-17.

The terms "effective amount" and "therapeutically effective amount" mean the amount of a therapy that is sufficient to reduce or ameliorate the severity and/or duration
20 of a disorder or one or more symptoms thereof; prevent the advancement of a disorder; cause regression of a disorder; prevent the recurrence, development, onset, or progression of one or more symptoms associated with a disorder; detect a disorder; or enhance or improve the prophylactic or therapeutic effect(s) of another therapy (*e.g.*, prophylactic or therapeutic agent).

25 The terms "patient" and "subject" mean an animal, such as a mammal, including a primate (*e.g.*, a human, a monkey, and a chimpanzee), a non-primate (for example, a cow, a pig, a camel, a llama, a horse, a goat, a rabbit, a sheep, a hamster, a guinea pig, a cat, a dog, a rat, a mouse, a whale), a bird and a fish. In an embodiment, the patient or subject is a human, such as a human being treated or assessed for a disease, disorder or
30 condition; a human at risk for a disease, disorder or condition; and/or a human having a disease, disorder or condition.

The term "sample" means a quantity of a substance. The term "biological sample" means a quantity of a substance obtained from a living thing or formerly living

thing. Such substances include, but are not limited to, blood, plasma, serum, urine, amniotic fluid, synovial fluid, endothelial cells, leukocytes, monocytes, other cells, organs, tissues, bone marrow, lymph nodes, joint tissue, synovium tissue, fibroblast-like synoviocytes and spleen.

5 The term "biological activity" means all inherent biological properties of a molecule.

 The terms "disease-modifying anti-rheumatic drug" and "DMARD" mean a drug or agent that modulates, reduces or treats the symptoms and/or progression associated with an immune system disease, including autoimmune diseases (*e.g.*, rheumatic
10 diseases), graft-related disorders and immunoproliferative diseases. The DMARD may be a synthetic DMARD (*e.g.*, a conventional synthetic disease modifying antirheumatic drug) or a biologic DMARD. For example, the DMARD used may be a methotrexate, a sulfasalazine (Azulfidine), a cyclosporine (Neoral®, Sandimmune®), a leflunomide (Arava®), a hydroxychloroquine (Plaquenil®), a Azathioprine (Imuran®), or a
15 combination thereof. In various embodiments, a DMARD is used to treat or control progression, joint deterioration, and/or disability associated with an autoimmune disorder, *e.g.*, RA.

 The term "polypeptide" means any polymeric chain of amino acids and encompasses native or artificial proteins, polypeptide analogs or variants of a protein
20 sequence, or fragments thereof. A polypeptide may be monomeric or polymeric. For an antigenic polypeptide, a fragment of a polypeptide optionally contains at least one contiguous or nonlinear epitope. The precise boundaries of the at least one epitope fragment can be confirmed using ordinary skill in the art.

 The term "variant" means a polypeptide that differs from a given polypeptide in
25 amino acid sequence by the addition, deletion, or conservative substitution of amino acids, but that retains the biological activity of the given polypeptide (*e.g.*, a variant TNF- α can compete with anti-TNF- α antibody for binding to TNF). A conservative substitution of an amino acid, *i.e.*, replacing an amino acid with a different amino acid of similar properties (*e.g.*, hydrophilicity and degree and distribution of charged regions) is
30 recognized in the art as typically involving a minor change. These minor changes can be identified, in part, by considering the hydropathic index of amino acids, as understood in the art (see, *e.g.*, Kyte et al. (1982) J.Mol. Biol. 157:105-132). The term "variant" encompasses a polypeptide or fragment thereof that has been differentially processed,

such as by proteolysis, phosphorylation, or other post-translational modification, yet retains its biological activity or antigen reactivity, *e.g.*, the ability to bind to TNF- α and IL-17. The term "variant" encompasses fragments of a variant unless otherwise contradicted by context.

5 The term "isolated protein" or "isolated polypeptide" is a protein or polypeptide that by virtue of its origin or source of derivation is not associated with naturally associated components that accompany it in its native state; is substantially free of other proteins from the same species; is expressed by a cell from a different species; or does not occur in nature. Thus, a protein or polypeptide that is chemically synthesized or
10 synthesized in a cellular system different from the cell from which it naturally originates is isolated from its naturally associated components. A protein or polypeptide may also be rendered substantially free of naturally associated components by isolation using protein purification techniques well known in the art.

15 The terms "IL-17" and "IL-17A" mean a cytokine secreted by activated T-cells, which acts as a potent mediator in immune responses by inducing immune signaling molecules in various tissues to recruit monocytes and neutrophils to the site of inflammation. IL-17 exists in a monomeric form and also in a dimeric form (*i.e.*, as an IL-17A/IL-17A homodimer and as a IL-17A/IL-17F heterodimer).

20 The term "human IL-17" ("hIL-17") means a monomeric and dimeric cytokine protein of human origin. The term "human IL-17" includes an IL-17 homodimeric protein comprising two 15 kD IL-17A proteins (hIL-17A/A) and an IL-17 heterodimeric protein comprising a 15 kD IL-17A protein and a 15 kD IL-17F protein ("hIL-17A/F") of human origin. The amino acid sequences of hIL-17A and hIL-17F are shown below. The term "hIL-17" includes recombinant hIL-17 (rhIL-17), which can be prepared by
25 standard recombinant expression methods.

Table 11: Human IL-17A Amino Acid Sequence

SEQ ID NO	123456789012345678901234567890123456789012345678901234567890
31	GITIPRNP GCPNSEDKNFPRTVMVNLNIHNRNTNTNPKRSSDYNNRSTSPWNLHRNEDPE RYP SVIWEAKCRHLGCINADGNVDYHMNSVPIQQEILVLRREPPHCPNSFRLEKILVSVG CTCVTPIVHHVA

Table 12: Human IL-17F Amino Acid Sequence

SEQ ID NO	123456789012345678901234567890123456789012345678901234567890
32	RKIPKVGHTFFQKPESCPPVPGGSMKLDIGIINENQRVSMRNIESRSTSPWNYTVTWD NRYPSEVVQAQCRNLGCINAQ GKEDISMNSVPIQQETLVVRRKHQGC SVSFQLEKVLVTV GCTCVTPVIHHVQ

The term "IL-17/TNF- α binding protein" means a bispecific binding protein (*e.g.*, DVD-Ig protein) that binds IL-17 and TNF- α . The relative positions of the TNF- α binding region and IL-17 binding region within the bispecific binding protein are not
 5 fixed (*e.g.*, VD1 or VD2 of the DVD-Ig protein) unless specifically specified herein.

The terms "tumor necrosis factor" and "TNF" mean a naturally occurring cytokine that is involved in normal inflammatory and immune responses. Elevated levels of TNF play an important role in pathologic inflammation. "TNF" and "TNF- α " are used interchangeably herein with the term "tumor necrosis factor". In various
 10 embodiments, the TNF- α is a mammalian TNF- α including, *e.g.*, human TNF- α .

The term "human TNF- α " ("hTNF- α ", or simply "hTNF") means a 17 kD secreted form and a 26 kD membrane associated form of a human cytokine, the biologically active form of which is composed of a trimer of noncovalently bound 17 kD molecules. The structure of hTNF- α is described further in, for example, Pennica *et al.*
 15 (1984) Nature 312:724-729; Davis *et al.* (1987) Biochem. 26:1322-1326; and Jones *et al.* (1989) Nature 338:225-228. The term hTNF- α includes recombinant human TNF- α ("rhTNF- α "). The amino acid sequence of hTNF- α is shown below.

Table 13: Human TNF- α Amino Acid Sequence

SEQ ID NO	123456789012345678901234567890123456789012345678901234567890
33	MSTESMIRDVELAEEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTLFCLLHFGVIGPQR EEFPRDLSLISPLAQAVRSSSRTPSDKPVAVHVVANPQAEGLQWLNRRANALLANGVELR DNQLVVPSEGLYLIYSQVLFKGQGCPSHVLTHITISRIAVSYQTKVNLLSAIKSPCQRE TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLDFAESGQVYFGIIAL

20

The terms "specific binding" and "specifically binding", in reference to the interaction of an antibody, a protein, or a peptide with a second chemical species, mean that the interaction is dependent upon the presence of a particular structure (*e.g.*, an antigenic determinant or epitope) on the chemical species. If an antibody is specific for
 25 epitope "A", in the presence of a molecule containing epitope A (or free, unlabeled epitope A) in which "A" is labeled, the antibody reduces the amount of labeled A bound to the antibody. The term "specific binding partner" means a member of a specific binding pair. The term "specific binding pair" includes two different molecules, which specifically bind to each other through chemical or physical means (*e.g.*, an antigen (or
 30 fragment thereof) and an antibody (or antigenically reactive fragment thereof)).

Therefore, in addition to antigen and antibody specific binding pairs of common immunoassays, other specific binding pairs can include biotin and avidin (or streptavidin), carbohydrates and lectins, complementary nucleotide sequences, effector and receptor molecules, cofactors and enzymes, enzyme inhibitors and enzymes, and the like. Furthermore, specific binding pairs can include members that are analogs of the original specific binding members, for example, an analyte-analog. Immunoreactive specific binding members include antigens, antigen fragments, and antibodies, including monoclonal and polyclonal antibodies as well as complexes, fragments, and variants (including fragments of variants) thereof, whether isolated or recombinantly produced.

The terms "specific" and "specificity" in the context of an interaction between members of a specific binding pair refer to the selective reactivity of the interaction.

The term "human antibody" means antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR3. However, the term "human antibody" does not include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

The term "recombinant human antibody" means human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell, antibodies isolated from a recombinant, combinatorial human antibody library, antibodies isolated from an animal that is transgenic for human immunoglobulin genes, or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies are subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

The term "CDR" means the complementarity determining region within antibody variable sequences. There are three CDRs in each of the variable regions of the heavy chain and the light chain, which are designated CDR1, CDR2, and CDR3, for each of the variable regions. The term "CDR set" means a group of three CDRs that occur in a single variable region (*i.e.*, VH or VL) of an antigen binding site. The exact boundaries of these CDRs have been defined differently according to different systems. The system described by Kabat (Kabat *et al.* (1987, 1991) Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Maryland) provides an unambiguous residue numbering system applicable to any variable region of an antibody and provides precise residue boundaries defining the three CDRs. These CDRs may be referred to as Kabat CDRs. Chothia and coworkers (Chothia and Lesk (1987) J. Mol. Biol. 196: 901-917 and Chothia *et al.* (1989) Nature 342: 877-883) found that certain sub-portions within Kabat CDRs adopt nearly identical peptide backbone conformations, despite having great diversity at the level of amino acid sequence. These sub-portions were designated as L1, L2, and L3 or H1, H2, and H3, where the "L" and the "H" designates the light chain and the heavy chains regions, respectively. These regions may be referred to as Chothia CDRs, which have boundaries that overlap with Kabat CDRs. Other boundaries defining CDRs overlapping with the Kabat CDRs have been described by Padlan *et al.* (1995) FASEB J. 9: 133-139 and MacCallum (1996) J. Mol. Biol. 262(5): 732-745). Still other CDR boundary definitions may not strictly follow one of the above systems, but nonetheless overlap with the Kabat CDRs, although they may be shortened or lengthened in light of prediction or experimental findings that particular residues or groups of residues or even entire CDRs do not significantly impact antigen binding. The methods used herein may utilize CDRs defined according to any of these systems, although certain embodiments use Kabat or Chothia defined CDRs.

The terms "Kabat numbering," "Kabat definition," and "Kabat labeling" mean a system of numbering amino acid residues which are more variable (*i.e.*, hypervariable) than other amino acid residues in the heavy and light chain variable regions of an antibody, or an antigen binding portion thereof (Kabat *et al.* (1971) Ann. NY Acad. Sci. 190: 382-391 and Kabat *et al.* (1991) "Sequences of Proteins of Immunological Interest, Fifth Edition", U.S. Department of Health and Human Services, NIH Publication No. 91-3242). For the heavy chain variable region, the hypervariable region ranges from amino acid positions 31 to 35 for CDR1, amino acid positions 50 to 65 for CDR2, and

amino acid positions 95 to 102 for CDR3. For the light chain variable region, the hypervariable region ranges from amino acid positions 24 to 34 for CDR1, amino acid positions 50 to 56 for CDR2, and amino acid positions 89 to 97 for CDR3.

The growth and analysis of extensive public databases of amino acid sequences of variable heavy and light regions over the past twenty years have led to the understanding of the typical boundaries between framework regions (FR) and CDR sequences within variable region sequences and enabled persons skilled in this art to accurately determine the CDRs according to Kabat numbering, Chothia numbering, or other systems. See, *e.g.*, Martin, "Protein Sequence and Structure Analysis of Antibody Variable Domains," *In* Kontermann and Dübel, eds., Antibody Engineering (Springer-Verlag, Berlin, 2001), Chapter 31, pages 432-433. A useful method of determining the amino acid sequences of Kabat CDRs within the amino acid sequences of variable heavy (VH) and variable light (VL) regions is provided below:

To identify a CDR-L1 amino acid sequence:

Starts approximately 24 amino acid residues from the amino terminus of the VL region;

Residue before the CDR-L1 sequence is always cysteine (C);

Residue after the CDR-L1 sequence is always a tryptophan (W) residue, typically Trp-Tyr-Gln (W-Y-Q), but also Trp-Leu-Gln (W-L-Q), Trp-Phe-Gln (W-F-Q), and Trp-Tyr-Leu (W-Y-L); and

Length is typically 10 to 17 amino acid residues.

To identify a CDR-L2 amino acid sequence:

Starts always 16 residues after the end of CDR-L1;

Residues before the CDR-L2 sequence are generally Ile-Tyr (I-Y), but also Val-Tyr (V-Y), Ile-Lys (I-K), and Ile-Phe (I-F); and

Length is always 7 amino acid residues.

To identify a CDR-L3 amino acid sequence:

Starts always 33 amino acids after the end of CDR-L2;

Residue before the CDR-L3 amino acid sequence is always a cysteine (C);

Residues after the CDR-L3 sequence are always Phe-Gly-X-Gly (F-G-X-G) (SEQ ID NO:34), where X is any amino acid; and

Length is typically 7 to 11 amino acid residues.

To identify a CDR-H1 amino acid sequence:

Starts approximately 31 amino acid residues from amino terminus of VH region and always 9 residues after a cysteine (C);

Residues before the CDR-H1 sequence are always Cys-X-X-X-X-X-X-X (SEQ ID NO:35), where X is any amino acid;

5 Residue after CDR-H1 sequence is always a Trp (W), typically Trp-Val (W-V), but also Trp-Ile (W-I), and Trp-Ala (W-A); and

Length is typically 5 to 7 amino acid residues.

To identify a CDR-H2 amino acid sequence:

Starts always 15 amino acid residues after the end of CDR-H1;

10 Residues before CDR-H2 sequence are typically Leu-Glu-Trp-Ile-Gly (L-E-W-I-G) (SEQ ID NO:36), but other variations also;

Residues after CDR-H2 sequence are Lys/Arg-Leu/Ile/Val/Phe/Thr/Ala-Thr/Ser/Ile/Ala (K/R-L/I/V/F/T/A-T/S/I/A); and

Length is typically 16 to 19 amino acid residues.

15 To identify a CDR-H3 amino acid sequence:

Starts always 33 amino acid residues after the end of CDR-H2 and always 3 after a cysteine (C);

Residues before the CDR-H3 sequence are always Cys-X-X (C-X-X), where X is any amino acid, typically Cys-Ala-Arg (C-A-R);

20 Residues after the CDR-H3 sequence are always Trp-Gly-X-Gly (W-G-X-G) (SEQ ID NO:37), where X is any amino acid; and

Length is typically 3 to 25 amino acid residues.

The term "neutralizing" means to render inactive activity, *e.g.*, the biological activity of an antigen (*e.g.*, the cytokines TNF- α and IL-17) when a binding protein specifically binds the antigen. Preferably, a neutralizing binding protein binds to human TNF- α and/or human IL-17 resulting in the inhibition of a biological activity of the cytokines. Preferably, the neutralizing binding protein binds TNF- α and IL-17 and reduces a biological activity of TNF- α and IL-17 by at least about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, or more. Inhibition of a biological activity of TNF- α and IL-17 by a neutralizing binding protein can be assessed by measuring one or more indicators of TNF- α and IL-17 biological activity well known in the art.

The term "activity" includes activities such as the binding specificity/affinity of an antibody for an antigen, e.g., an anti-hTNF- α and anti-hIL-17 bispecific antibody that binds to TNF- α and IL-17.

The terms "label" and "detectable label" mean a moiety attached to a specific binding partner, such as an antibody or an analyte, e.g., to render the reaction between two specific binding partners (*i.e.*, a specific binding pair) detectable. The specific binding partner so labeled is referred to as "detectably labeled". Thus, the term "labeled binding protein" means a protein with a label incorporated that provides for the identification of the binding protein or the ligand to which it binds. In an embodiment, the label is a detectable label that can produce a signal that is detectable by visual or instrumental means, e.g., incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin or streptavidin (e.g., streptavidin containing a fluorescent label or enzymatic activity that can be detected by optical or colorimetric methods). Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides (e.g., ^3H , ^{14}C , ^{35}S , ^{90}Y , ^{99}Tc , ^{111}In , ^{125}I , ^{131}I , ^{177}Lu , ^{166}Ho , or ^{153}Sm), chromogens, fluorescent labels (e.g., FITC, rhodamine, lanthanide phosphors), enzymatic labels (e.g., horseradish peroxidase, luciferase, alkaline phosphatase), chemiluminescent labels, biotinyl groups, predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags), and magnetic agents (e.g., gadolinium chelates). Representative examples of labels commonly employed for immunoassays include moieties that produce light, e.g., acridinium compounds, and moieties that produce fluorescence, e.g., fluorescein. In this regard, the moiety itself may not be detectably labeled but may become detectable upon reaction with yet another moiety. Use of the term "detectably labeled" is intended to encompass the latter type of detectable labeling.

The term "agent" means a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials. The therapeutic or cytotoxic agents include, but are not limited to, pertussis toxin, taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, teniposide, vincristine, vinblastine, colchicine, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs

or homologs thereof. When employed in the context of an immunoassay, a binding protein conjugate may be a detectably labeled antibody, which is used as the detection antibody.

The term "polynucleotide" means a polymer of two or more nucleotides, e.g., ribonucleotides or deoxynucleotides or a modified form of nucleotide. The term includes single and double stranded forms of DNA.

The term "isolated polynucleotide" means a polynucleotide (e.g., of genomic, cDNA, or synthetic origin, or some combination thereof) that, by virtue of its origin, is not associated with all or a portion of a polynucleotide with which the polynucleotide is found in nature; is operably linked to a polynucleotide that it is not linked to in nature; or does not occur in nature as part of a larger sequence.

The term "vector" means a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional nucleic acid segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked ("recombinant expression vectors" or "expression vectors"). In general, expression vectors are often in the form of plasmids. Vectors may also be viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses). Standard techniques may be used for recombinant DNA, oligonucleotide synthesis, tissue culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques may be performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures may be generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. See e.g., Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2nd ed. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989).

The term "modulator" means a compound capable of changing or altering an activity or function of a molecule of interest (*e.g.*, the biological activity of hTNF- α and hIL-17). For example, a modulator may cause an increase or decrease in the magnitude of a certain activity or function of a molecule compared to the magnitude of the activity or function observed in the absence of the modulator. In certain embodiments, a modulator is an inhibitor, which decreases the magnitude of at least one activity or function of a molecule. Exemplary inhibitors include, but are not limited to, proteins, peptides, antibodies, peptibodies, carbohydrates or small organic molecules. Peptibodies are described, *e.g.*, in PCT Publication No. WO01/83525.

The term "agonist" means a modulator that, when contacted with a molecule of interest, causes an increase in the magnitude of a certain activity or function of the molecule compared to the magnitude of the activity or function observed in the absence of the agonist. Particular agonists of interest may include, but are not limited to, TNF- α and IL-17 polypeptides, nucleic acids, carbohydrates, or any other molecule that binds to hTNF- α and hIL-17.

The term "multivalent binding protein" means a binding protein comprising two or more antigen binding sites. A multivalent binding protein is preferably engineered to have three or more antigen binding sites, and is generally not a naturally occurring antibody. The term "multispecific binding protein" means a binding protein capable of binding two or more related or unrelated targets. "Dual variable domain" ("DVD") binding proteins of the invention comprise two or more antigen binding sites and are tetravalent or multivalent binding proteins. DVDs may be monospecific, *i.e.*, capable of binding one antigen, or multispecific, *i.e.*, capable of binding two or more antigens. A DVD binding protein comprising two heavy chain DVD polypeptides and two light chain DVD polypeptides is referred to as a "DVD immunoglobulin" or "DVD-Ig". Each half of a DVD-Ig comprises a heavy chain DVD polypeptide and a light chain DVD polypeptide, and two or more antigen binding sites. Each binding site comprises a heavy chain variable domain and a light chain variable domain with a total of six CDRs involved in antigen binding per antigen binding site. See U.S. Patent Nos. 7, 612,181; 8,258,268, and 8,779,101, which are incorporated by reference herein in their entireties.

Multivalent binding proteins in various embodiments include bispecific molecules which can be generated using a number of different methods (Spiess *et al.* (2015) *Mol. Immunol.* pii: S0161-5890). In various embodiments, bispecific molecules

comprise Triomab quadroma bispecifics / removab bispecifics, bispecific T cell engagers, tetravalent bispecific tandem diabodies, crossMabs, DARTTMs, innovative multimers, DutaMabs, asymmetric bispecific antibodies, two-in-one antibodies, Fabsc antibodies, asymmetric bispecific IgG4s, VHHs / nanobodiesTM, cross-over dual variable immunoglobulins, biconics and the like. Multivalent binding proteins in various embodiments include full-length antibodies that are generated by quadroma technology (see Milstein and Cuello (1983) *Nature* 305: 537-540), by chemical conjugation of two different monoclonal antibodies (see Staerz *et al.* (1985) *Nature* 314: 628-631), or by knob-into-hole or similar approaches which introduces mutations in the Fc region (see Holliger *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90(14): 6444-6448), resulting in multiple different immunoglobulin species of which only one is the functional bispecific antibody.

For example, an anti-TNF / anti-IL-17 bispecific can be prepared using any number of formats and techniques: fusion protein, bispecific nanobody, a cross mab, diabody, and DVD-Ig formats (See European patent application EP 2597102A1, International Application Nos. WO2012156219 and WO2014137961, Fischer *et al.* (2015) *Arthrit. Rheumatol.* 67: 51-62. doi:10.1002/art.38896, U.S. Publication No. US20140079705, and U.S. Patent No. 8,779,101, which are incorporated by reference in its entirety). The terms “single chain dual variable domain immunoglobulin protein” or “scDVD-Ig protein” or scFvDVDIg protein” refer to the antigen binding fragment of a DVD molecule that is analogous to an antibody single chain Fv fragment. scDVD-Ig proteins are described in U.S. Publication Nos. US2014-0243228 and US2014-0221621, which are incorporated herein by reference in their entirety. The scDVD-Ig proteins are generally of the formula VH1-(X1)_n-VH2-X2-VL1-(X3)_n-VL2, where VH1 is a first antibody heavy chain variable domain, X1 is a linker with the proviso that it is not a constant domain, VH2 is a second antibody heavy chain variable domain, X2 is a linker, VL1 is a first antibody light chain variable domain, X3 is a linker with the proviso that it is not a constant domain, VL2 is a second antibody light chain variable domain, and n is 0 or 1, where the VH1 and VL1, and the VH2 and VL2 respectively combine to form two functional antigen binding sites.

Various aspects of the invention are described in further detail in the following paragraphs.

Diagnostic Methods

The level of expression of a biomarker in a sample obtained from a subject may be assayed by any of a wide variety of techniques and methods, which transform the biomarker within the sample into a moiety that can be detected and/or quantified. Non-limiting examples of such methods include analyzing the sample using immunological methods for detection of proteins, protein purification methods, protein function or activity assays, nucleic acid hybridization methods, nucleic acid reverse transcription methods, and nucleic acid amplification methods, immunoblotting, Western blotting, Northern blotting, electron microscopy, mass spectrometry, *e.g.*, MALDI-TOF and SELDI-TOF, immunoprecipitation, immunofluorescence, immunohistochemistry, enzyme linked immunosorbent assays (ELISAs), *e.g.*, amplified ELISA, quantitative blood based assays, *e.g.*, serum ELISA, quantitative urine based assays, flow cytometry, Southern hybridizations, array analysis, and the like, and combinations or sub-combinations thereof.

In one embodiment, the level of expression of the biomarker in a sample is determined by detecting a transcribed polynucleotide, or portion thereof, *e.g.*, mRNA, or cDNA, of the biomarker gene. RNA may be extracted from cells using RNA extraction techniques including, for example, using acid phenol/guanidine isothiocyanate extraction (RNAzol B; Biogenesis), RNeasy RNA preparation kits (Qiagen) or PAXgene (PreAnalytix, Switzerland). Typical assay formats utilizing ribonucleic acid hybridization include nuclear run-on assays, RT-PCR, quantitative PCR analysis, RNase protection assays, Northern blotting and *in situ* hybridization. Other suitable systems for mRNA sample analysis include microarray analysis (*e.g.*, using Affymetrix's microarray system or Illumina's BeadArray Technology).

In one embodiment, the level of expression of the biomarker is determined using a nucleic acid probe. The term "probe", as used herein, refers to any molecule that is capable of selectively binding to a specific biomarker and/or is useful for identifying the presence or properties of the biomarker. Probes can be synthesized by one of skill in the art, or derived from appropriate biological preparations. Probes can be specifically designed to be labeled, by addition or incorporation of a label. Examples of molecules that can be utilized as probes include, but are not limited to, RNA, DNA, proteins, antibodies, and organic molecules.

As indicated above, isolated mRNA can be used in hybridization or amplification assays that include, but are not limited to, Southern or Northern analyses, polymerase chain reaction (PCR) analyses and probe arrays. One method for the determination of mRNA levels involves contacting the isolated mRNA with a nucleic acid molecule (probe) that can hybridize to the biomarker mRNA. The nucleic acid probe can be, for example, a full-length cDNA, or a portion thereof, such as an oligonucleotide of at least about 7, 10, 15, 20, 25, 30, 35, 40, 45, 50, 100, 250 or about 500 nucleotides in length and sufficient to specifically hybridize under appropriate hybridization conditions to the biomarker genomic DNA. In a particular embodiment, the probe will bind the biomarker genomic DNA under stringent conditions. Such stringent conditions, for example, hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45° C., followed by one or more washes in 0.2X SSC, 0.1% SDS at 50-65° C., are known to those skilled in the art and can be found in Current Protocols in Molecular Biology, Ausubel *et al.*, eds., John Wiley & Sons, Inc. (1995), sections 2, 4, and 6, the teachings of which are hereby incorporated by reference herein. Additional stringent conditions can be found in Molecular Cloning: A Laboratory Manual, Sambrook *et al.*, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (1989), chapters 7, 9, and 11, the teachings of which are hereby incorporated by reference herein.

In one embodiment, the mRNA is immobilized on a solid surface and contacted with a probe, for example by running the isolated mRNA on an agarose gel and transferring the mRNA from the gel to a membrane, such as nitrocellulose. In an alternative embodiment, the probe(s) are immobilized on a solid surface, for example, in an Affymetrix gene chip array, and the probe(s) are contacted with mRNA. A skilled artisan can readily adapt mRNA detection methods for use in determining the level of the biomarker mRNA.

Other known methods for detecting the biomarker at the protein level include methods such as electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), hyperdiffusion chromatography, and the like, or various immunological methods such as fluid or gel precipitation reactions, immunodiffusion (single or double), immunoelectrophoresis, radioimmunoassay (RIA), immunofluorescent assays, and Western blotting.

The present invention is further illustrated by the following examples which should not be construed as further limiting. The contents of all references, patents and

published patent applications cited throughout this application, as well as the Figures are expressly incorporated herein by reference in their entirety.

EXAMPLES

5 **Example 1: Efficacy of Anti-TNF- α /IL-17 DVD-Ig Protein in a Mouse Collagen Induced Arthritis Model**

Anti-murine TNF antibody 8C11, anti-murine IL-17 antibody MAB421, both anti-TNF and anti-IL-17 antibodies, or an anti-mouse TNF/IL-17 DVD-Ig protein 8C11/10F7M11 (Table 18) were tested in a mouse collagen induced arthritis (CIA) model to determine whether dual neutralization of TNF and IL-17 with a bispecific molecule utilizing dual variable domain technology would confer efficacy in an arthritis model with the intended pharmacologic activity in the joint (Figure 1, panels A-E). Figure 2 shows a schematic of an anti-murine TNF/IL-17 DVD-Ig protein, composed of 8C11 (anti-murine TNF antibody) and 10F7M11 (anti-murine IL-17 antibody) sequences. The amino acid sequences of the variable domains and CDRs of the antibodies and DVD-Ig proteins used in these studies are provided below in Tables 14-17.

Table 14: Sequences of 8C11 and 10F7M11 Antibody Variable Domains

SEQ ID NO	Clone	Variable Domain	1234567890123456789012345678901234567890
1	8C11-VH	VH	EFQLQQSGPELVKPGASVRISCKASGYSTFDYNNMNWVKQS NGKSLEWVGVINPNYGSSTYNQKFKGKATLTVDQSSSTAY MQLNSLTSEDSAVYYCARKWGQ ~~LGRGFFD~~ VWGTGTTVTVS S
2	8C11-VL	VL	QIVLSQSPAILASAPGEKVTMTCSRASSSVSYMHWFQQKPG SSPKPWIYATSNLASGVPARFSGSGSGTSYSLTISRVEAE DAATYYCQWSSSPLTFGAGTKLELKR
3	10F7M11-VH	VH	QVQLQQSGAELVRPGTSVTLSCASGYIFTDYEIHWVKQT PVHGLEWIGVNDPESGGTFYNQKFDGKAELTADKSSSTAY MELRSLTSEDSGVYYCTRYRYYESFYGMDYWGQGSITVS S
4	10F7M11-VL	VL	QIVLTQSPAIMASAPGEKVTMTCSASSSISYIYWFQQKPG TSPKRWIYATFELASGVPARFSGSGSGTSYSLTISSMEAE DAATYYCHQRSSYPWTFGGGSKLEIKR

Table 15: Sequences of anti-TNF- α Antibody 8C11 CDRs

VH 8C11 CDR Set	
VH 8C11 CDR-H1	Residues 31-35 of SEQ ID NO:1
VH 8C11 CDR-H2	Residues 50-66 of SEQ ID NO:1

VH 8C11 CDR-H3	Residues 99-109 of SEQ ID NO:1
VH 8C11 CDR Set	
VL 8C11 CDR-L1	Residues 24-33 of SEQ ID NO:2
VL 8C11 CDR-L2	Residues 49-55 of SEQ ID NO:2
VL 8C11 CDR-L3	Residues 88-96 of SEQ ID NO:2

Table 16: Sequences of Anti-IL-17 Antibody 10F7M11 CDRs

VH 10F7M11 CDR Set	
VH 10F7M11 CDR-H1	Residues 31-35 of SEQ ID NO:3
VH 10F7M11 CDR-H2	Residues 50-66 of SEQ ID NO:3
VH 10F7M11 CDR-H3	Residues 99-110 of SEQ ID NO:3
VH 10F7M11 CDR Set	
VL 10F7M11 CDR-L1	Residues 24-33 of SEQ ID NO:4
VL 10F7M11 CDR-L2	Residues 49-55 of SEQ ID NO:4
VL 10F7M11 CDR-L3	Residues 88-96 of SEQ ID NO:4

Table 17: Sequences of the Anti-mouse TNF/IL-17 DVD-Ig Protein

Name	SEQ ID NO	1234567890123456789012345678901234567890
DVD HEAVY VARIABLE 8C11-linker- 10F7M11- DVD	5	EFQLQQSGPELVKPGASVRISCKASGYSFTDYNMNWVKQS NGKSLWVGVINPNYGSSTYNQKFKGKATLTVDQSSSTAY MQLNSLTSEDSAVYYCARKWGQLGRGFFDVWGTGTTVTVS SGGGSGGGGSQVQLQQSGAELVRPGTSVTLSCASGYIF TDYIEIHWVKQTPVHGLEWIGVNDPESGGTFYNQKFDGKAE LTADKSSSTAYMELRSLTSEDSGVYYCTRYRYESFYGM YWGQTSITVSS
8C11 VH	1	EFQLQQSGPELVKPGASVRISCKASGYSFTDYNMNWVKQS NGKSLWVGVINPNYGSSTYNQKFKGKATLTVDQSSSTAY MQLNSLTSEDSAVYYCARKWGQLGRGFFDVWGTGTTVTVS S
linker	6	GGGGSGGGGS
10F7M11 VH	3	QVQLQQSGAELVRPGTSVTLSCASGYIFTDYIEIHWVKQT PVHGLEWIGVNDPESGGTFYNQKFDGKAELTADKSSSTAY MELRSLTSEDSGVYYCTRYRYESFYGM DYWGQTSITVS S
CH	7	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHPKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGG PSVFLFPPKPKDTLMI SRTPEVTCVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPV LDSGGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYT QKSLSLSPGK

DVD LIGHT VARIABLE 8C11-linker- 10F7M11- DVD	8	QIVLSQSPAILLSASPGEKVTMTCRASSSVSYMHWFQOKPG SSPKPWYIYATSNLASGVPARFSGSGSGTSYSLTISRVEAE DAATYYCQOWSSSPLTFGAGTKLELKRGGSGGGSGQIVL TQSPAIMSASPGEKVTMTCSASSISYIYWFQOKPGTSPK RWIYATFELASGVPARFSGSGSGTSYSLTISMEADAAT YYCHQRSSYPWTFGGGSKLEIKR
8C11 VL	2	QIVLSQSPAILLSASPGEKVTMTCRASSSVSYMHWFQOKPG SSPKPWYIYATSNLASGVPARFSGSGSGTSYSLTISRVEAE DAATYYCQOWSSSPLTFGAGTKLELKR
linker	9	GGSGGGGSG
10F7M11 VL	4	QIVLTQSPAIMSASPGEKVTMTCSASSISYIYWFQOKPG TSPKRWIYATFELASGVPARFSGSGSGTSYSLTISMEAE DAATYYCHQRSSYPWTFGGGSKLEIKR
CL	10	TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKSFNRGEC

Table 18: Source And Binding Information Regarding Anti-TNF Antibody, Anti-IL-17 Antibody And Anti-TNF/IL-17 DVD-Ig Protein

	Clone name	Source	Isotype	IC ₅₀ (nM)		Dose (mg/kg)	
				IL-17	TNF	21 day CIA	7 day CIA
Anti-TNF	8C11	AbbVie	Mouse IgG2c		2	12	6
Anti-IL-17	MAB421	R&D	Rat IgG2a	0.7		12	6
Anti-TNF/IL-17 DVD-Ig protein	8C11/10F7M11	AbbVie	Mouse IgG2a	0.13	2	16	0.1-10

- 5 Male DBA/1J mice were injected intradermally (i.d.) at the base of the tail with 100μL of an emulsion containing 100 μg of type II bovine collagen dissolved in 0.1N acetic acid and 100μL of Complete Freund's Adjuvant containing 100μg of Mycobacterium Tuberculosis H37Ra. Mice were boosted 21 days later intraperitoneally (i.p.) with 1.0 mg zymosan A in 200 μL of phosphate buffered saline (PBS). Disease onset occurred within 3 days of the boost. Mice were monitored for arthritis daily for the first week and monitored three times per week thereafter. The swelling of each paw was scored using a caliper. Animals were treated twice per week (2x/week) with 16 mg/kg i.p. injection of the 8C11/10F7-DVD-Ig protein, which has specificity for mouse TNF-α and IL-17. Mice receiving the anti-TNF-α/IL-17 DVD-Ig protein had significantly reduced paw swelling over the 21 days of disease compared to animals
- 10
- 15

receiving vehicle control (PBS). Data show that dual neutralization of TNF and IL-17 with a bispecific molecule was efficacious in an arthritis model.

Example 2. Metabolites and Enzymes of Arginine Metabolic Pathway and Urea Cycle as Biomarkers of ABT-122 Treatment.

ABT-122, a dual variable domain immunoglobulin (DVD-Ig) that neutralizes both TNF and IL-17, is currently in clinical trials for the treatment of rheumatoid arthritis (RA) and psoriatic arthritis (PsA). RA is driven largely by inflammatory processes, but changes in metabolic profiles have been reported in the synovial fluid and serum from RA patients, including metabolites within the urea and TCA cycles as well as lipid, glycolysis and amino acid metabolism. These changes in metabolic pathways may also contribute to pathogenesis of inflammatory disease as many of the alterations appear to be specific to different disease states. See Young *et al.* (2013) *Arthrit. Rheumat.* 65(8):2015-2023; Madsen *et al.* (2012) *J. Proteome Res.* 11:3796-3804; Madsen *et al.* (2011) *Arthrit. Res. Ther.* 13:R19; Jiang *et al.* (2013) *J. Proteome Res.* 12(8):3769-3779; and Kim *et al.* (2014) *PLOS One* 9(6):e97501).

In an effort to understand the pathways that are important in disease as well as with combined treatment of anti-TNF and anti-IL-17, gene array analysis was performed on paw homogenates from the murine CIA model seven days after initiation of therapies comprising anti-TNF antibody, anti-IL-17 antibody, or both antibodies. Through gene pathway analysis with Ingenuity software (Redwood City, CA), the arginine degradation (I and IV) pathways and urea cycles were identified as being significantly regulated only by the combination anti-TNF + anti-IL-17 treatment ($p < 0.003$ and at least two regulated transcripts) and not by the treatment with anti-TNF or anti-IL-17 alone. The gene expression changes primarily driving this were those of arginase I and arginase II, both of which were decreased with the combination anti-TNF and anti-IL-17 treatment (Figure 3).

To explore whether changes in the arginase pathway could be observed in humans, we evaluated serum from RA patients and healthy subjects for arginase I and arginase II levels by ELISA and arginine, ornithine, citrulline, proline as well as symmetric and asymmetric dimethylarginine (SDMA and ADMA, respectively) levels by mass spectrometry. Elevations in arginase II, but not arginase I (Figure 4), were observed, which are consistent with previous reports of arginase II or arginase I activity

elevations in serum of RA patients. See Moncada (2002) J. Rheumatol. 29(11):2261-5; Huang *et al.* (2001) Kaohsiung J. Med. Sci. 17(7):358-63. These observations were extended to also show elevations in serum arginine, but not ornithine or citrulline, other metabolites of the urea cycle, by mass spectrometry (Figure 5). Proline, SDMA and ADMA levels were also not altered between healthy and RA patients. However, the elevations in arginase II and arginine serum levels in RA patients are similarly elevated in RA patients receiving anti-TNF treatment (Figures 4 and 5) suggesting that anti-TNF treatment alone is not sufficient to alter the expression of arginase II or arginine.

As arginase is expressed in synovial fibroblasts, the effects of recombinant TNF and IL-17 on gene expression of enzymes in the arginase pathway and urea cycle from FLS cultures were evaluated. In vitro stimulation of the FLS cells with recombinant TNF and IL-17, but not individual cytokines, dramatically regulated gene expression of several enzymes in the urea cycle in FLS. Expression of arginase II, argininosuccinate lyase and nitric oxide synthetase (NOS) 1 are all upregulated by at least 3 fold whereas argininosuccinate synthetase 1 is inhibited more than 10 fold (Figure 6). NOS 2 and arginase I were relatively unaffected by the combination TNF and IL-17 cytokine treatment, although they were elevated by starvation conditions in the cultures as a positive control (Figure 7).

The cooperative regulation by TNF and IL-17 of the arginase metabolic pathway and urea cycle within inflamed paws in a pre-clinical model of arthritis, as well as in human FLS cells, suggests that metabolites and enzymes of these pathways are regulated specifically by this combination of cytokines and thus, could be used as biomarkers of response to combination anti-TNF and anti-IL-17. In addition, arginase and arginine are elevated in RA patients compared to healthy controls, but these elevated levels are not altered by anti-TNF treatment alone, further suggesting that combination anti-TNF and anti-IL-17 may be necessary to alter these biomarkers.

Incorporation by Reference

The contents of all cited references (including literature references, patents, patent applications, databases and websites) that may be cited throughout this application are hereby expressly incorporated by reference in their entirety for any purpose, as are the references cited therein. The practice of the present invention will employ, unless

otherwise indicated, conventional techniques of immunology, molecular biology and cell biology, which are well known in the art.

Equivalents

5 The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting of the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of
10 equivalency of the claims are therefore intended to be embraced herein.

CLAIMS

1. A method of monitoring or calibrating a dosage in a subject being treated for rheumatoid arthritis with a combination therapy comprising an anti-TNF treatment and an anti-IL-17 treatment, the method comprising the steps of
 - a) administering to the subject a first dose of a combination therapy comprising an anti-TNF treatment and an anti-IL-17 treatment;
 - b) determining a modulation of expression of one or more biomarkers in a sample from the subject, wherein the one or more biomarkers are components associated with the urea cycle;and
 - c) administering a second dose of the combination therapy, wherein the second dose is determined depending on the relative abundance of the one or more biomarkers in the subject sample in response to the first dose.
2. The method of claim 1, wherein the biomarkers are selected from the group consisting of arginine, arginase II, argininosuccinate lyase, nitric oxide synthetase 1 and argininosuccinate synthetase 1.
3. The method of claim 2, wherein the determination of modulation of expression of the one or more biomarkers in the sample from the subject comprises
 - i) detecting the interaction of one or more binding moieties that specifically bind to the one or more biomarkers or nucleic acids expressing the one or more biomarkers, thereby detecting the abundance of the one or more biomarkers in the subject sample; and
 - ii) obtaining a relative abundance of the one or more biomarkers in the subject sample by comparison to a baseline abundance of the biomarker.
4. The method of claim 3, wherein the second dose is equal to or greater than the first dose when the one or more biomarkers are selected from the group consisting of arginase II, argininosuccinate lyase and nitric oxide synthetase 1, and wherein the relative abundance of the one or more biomarkers in the subject sample in response to the first dose is greater when compared to the baseline abundance of the one or more biomarkers.

5. The method of claim 3, wherein the second dose is equal to or greater than the first dose when the one or more biomarkers is argininosuccinate synthetase 1 and wherein the relative abundance of the argininosuccinate synthetase 1 in the subject sample in response to the first dose is less when compared to the baseline abundance of the argininosuccinate synthetase 1.
6. The method of claim 3, wherein the second dose is less than the first dose or treatment is discontinued when one or more biomarkers are selected from the group consisting of arginase II, argininosuccinate lyase and nitric oxide synthetase 1, and wherein the relative abundance of the one or more biomarkers in the subject sample in response to the first dose is less when compared to the baseline abundance of the one or more biomarkers.
7. The method of claim 6, wherein the second dose is less than the first dose or treatment is discontinued when one or more biomarkers is argininosuccinate synthetase 1 and wherein the relative abundance of the argininosuccinate synthetase 1 in the subject sample in response to the first dose is greater when compared to the baseline abundance of the argininosuccinate synthetase 1.
8. The method of claim 1, wherein the second dose is equal to or greater than the first dose when the one or more biomarkers is arginine, and wherein the relative abundance of the arginine in the subject sample in response to the first dose is greater when compared to the baseline abundance of arginine.
9. The method of claim 1, wherein the second dose is less than the first dose or treatment is discontinued when one or more biomarkers is arginine, and wherein the relative abundance of the arginine in the subject sample in response to the first dose is less when compared to the baseline abundance of arginine.
10. Use of a combination therapy for treating a subject suffering from rheumatoid arthritis, the use comprising the steps of administering a dose of a combination therapy comprising an anti-TNF treatment and an anti-IL-17 treatment to the subject, wherein a

sample from the subject comprises an abundance of one or more biomarkers, wherein the one or more biomarkers are selected from the group consisting of arginase II, argininosuccinate lyase and nitric oxide synthetase 1, and wherein the relative abundance of the one or more biomarkers in the subject sample is greater when compared to a baseline abundance of the one or more biomarkers.

11. Use of a combination therapy for treating a subject suffering from rheumatoid arthritis, the use comprising the steps of administering a dose of a combination therapy comprising an anti-TNF treatment and an anti-IL-17 treatment to the subject, wherein a sample from the subject comprises an abundance of arginine, and wherein the relative abundance of arginine in the subject sample is greater when compared to a baseline abundance of the arginine.

12. Use of a combination therapy for treating a subject suffering from rheumatoid arthritis, the use comprising the steps of administering a dose of a combination therapy comprising an anti-TNF treatment and an anti-IL-17 treatment to the subject, wherein a sample from the subject comprises an abundance of argininosuccinate synthetase 1, and wherein the relative abundance of argininosuccinate synthetase 1, in the subject sample is less when compared to a baseline abundance of the argininosuccinate synthetase 1.

13. A method of screening a subject for rheumatoid arthritis, the method comprising the steps of

a) determining a modulation of expression of one or more biomarkers in a sample from the subject, wherein the one or more biomarkers are selected from the group consisting of arginase II, argininosuccinate lyase, nitric oxide synthetase (NOS) 1 and argininosuccinate synthetase 1;

b) detecting the interaction of one or more binding moieties that specifically bind to the one or more biomarkers or nucleic acids expressing one or more biomarkers, thereby detecting the abundance of the one or more biomarkers in the subject sample; and

c) obtaining a relative abundance of the one or more biomarkers in the subject sample by comparison to a baseline abundance of the one or more biomarker; wherein the subject has an increased risk of an inflammatory disorder when the

abundance of the one or more biomarkers is modulated.

14. The method of any of claims 1-13, wherein the baseline abundance of the biomarker is the abundance of the biomarker in a healthy subject.

15. The method of claims 14, wherein the healthy subject is not experiencing rheumatoid arthritis.

16. The method of any of claims 1-15, wherein the baseline abundance of the biomarker is the average abundance of the biomarker in two or more healthy subjects.

17. The method of any of claims 1-16, wherein the baseline abundance of the biomarker is the abundance of the biomarker in the treated subject before the subject experienced rheumatoid arthritis.

18. The method of any of claims 1-17, wherein the baseline abundance of the biomarker is the abundance of the biomarker in the treated subject before the subject was experiencing symptoms of rheumatoid arthritis.

19. The method of any of claims 1-18, wherein the sample comprises joint tissue.

20. The method of claim 19, wherein the joint tissue comprises synovium tissue or synovial fluid.

21. The method of claim 20, wherein the synovium tissue comprises fibroblast-like synoviocytes.

22. A method of decreasing the abundance of arginine in serum of a subject comprising administering to the subject an effective amount of a combination therapy comprising an anti-TNF treatment and an anti-IL-17 treatment.

23. A kit comprising of one or more binding moieties that specifically bind to one or more gene products or nucleic acids expressing the gene products selected from the

group consisting of arginase II, argininosuccinate lyase, nitric oxide synthetase (NOS) 1 and argininosuccinate synthetase 1.

24. The kit of claim 23, wherein the kit comprises two or more binding moieties.

25. The kit of claim 23, further comprising means for the detection of the abundance of arginine.

26. A method of determining a modulation of expression of one or more biomarkers in a sample from a subject with rheumatoid arthritis, wherein the one or more biomarkers are selected from the group consisting of arginase II, argininosuccinate lyase, nitric oxide synthetase (NOS) 1 and argininosuccinate synthetase 1, the method comprising the steps of

a) detecting the interaction of one or more binding moieties that specifically bind to the one or more biomarkers or nucleic acids expressing the biomarkers, thereby detecting the abundance of the one or more biomarkers in the subject sample; and

b) obtaining a relative abundance of the one or more biomarkers in the subject sample by comparison to a baseline abundance of the one or more biomarker.

27. The method of any of claim 26, wherein the baseline abundance of the biomarker is the abundance of the biomarker in a healthy subject.

28. The method of claims 27, wherein the healthy subject is not experiencing rheumatoid arthritis.

29. The method of any of claims 26-28, wherein the baseline abundance of the biomarker is the average abundance of the biomarker in two or more healthy subjects.

30. The method of any of claims 26-29, wherein the baseline abundance of the biomarker is the abundance of the biomarker in the treated subject before the subject experienced rheumatoid arthritis.

31. The method of any of claims 26-30, wherein the baseline abundance of the

biomarker is the abundance of the biomarker in the treated subject before the subject was experiencing symptoms of rheumatoid arthritis.

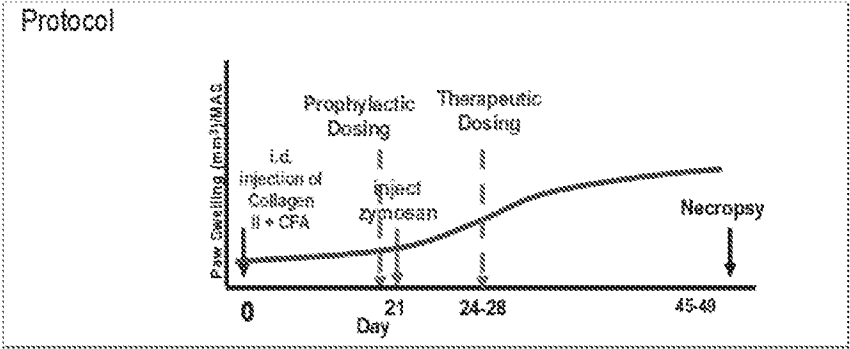
32. The method of any of claims 26-31, wherein the sample comprises joint tissue.

33. The method of claim 32, wherein the joint tissue comprises synovium tissue or synovial fluid.

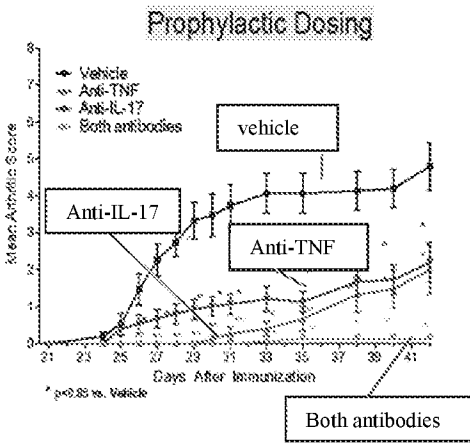
34. The method of claim 33, wherein the synovium tissue comprises fibroblast-like synoviocytes.

Figure 1

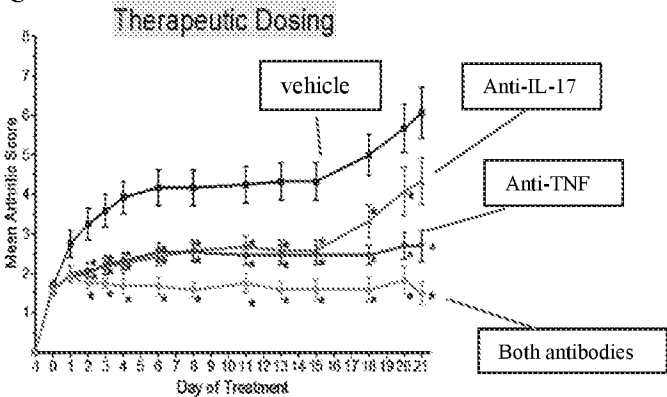
A



B



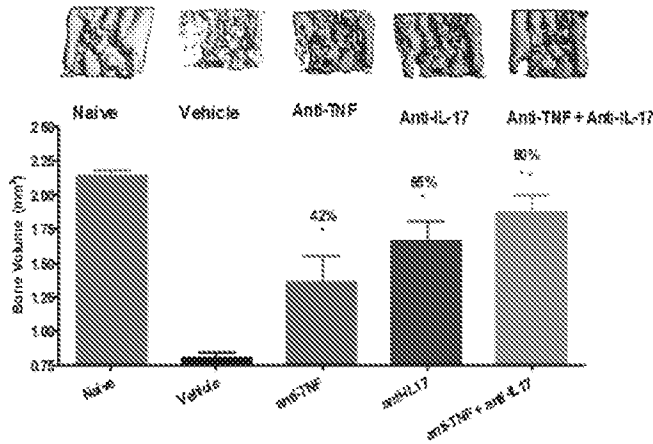
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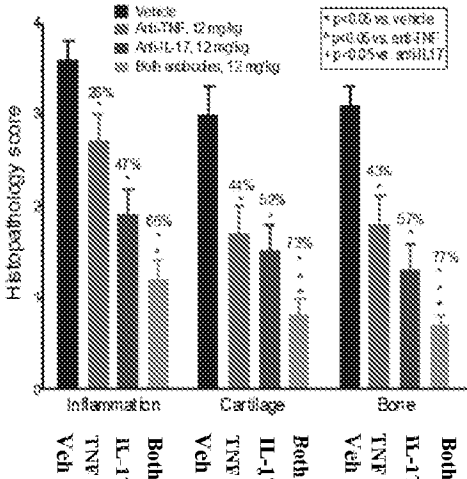
Micro-CT analysis of tarsal bone

Histological analysis of rear paw

D

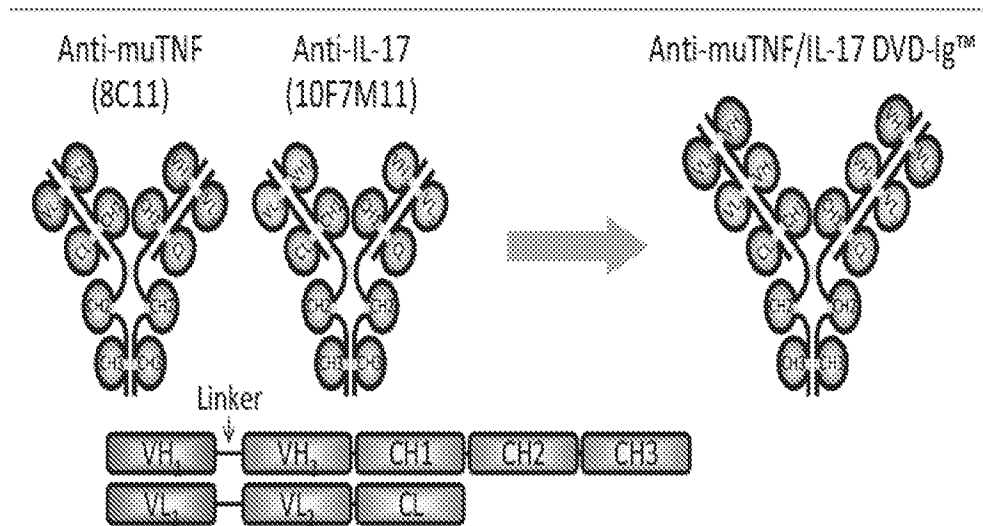


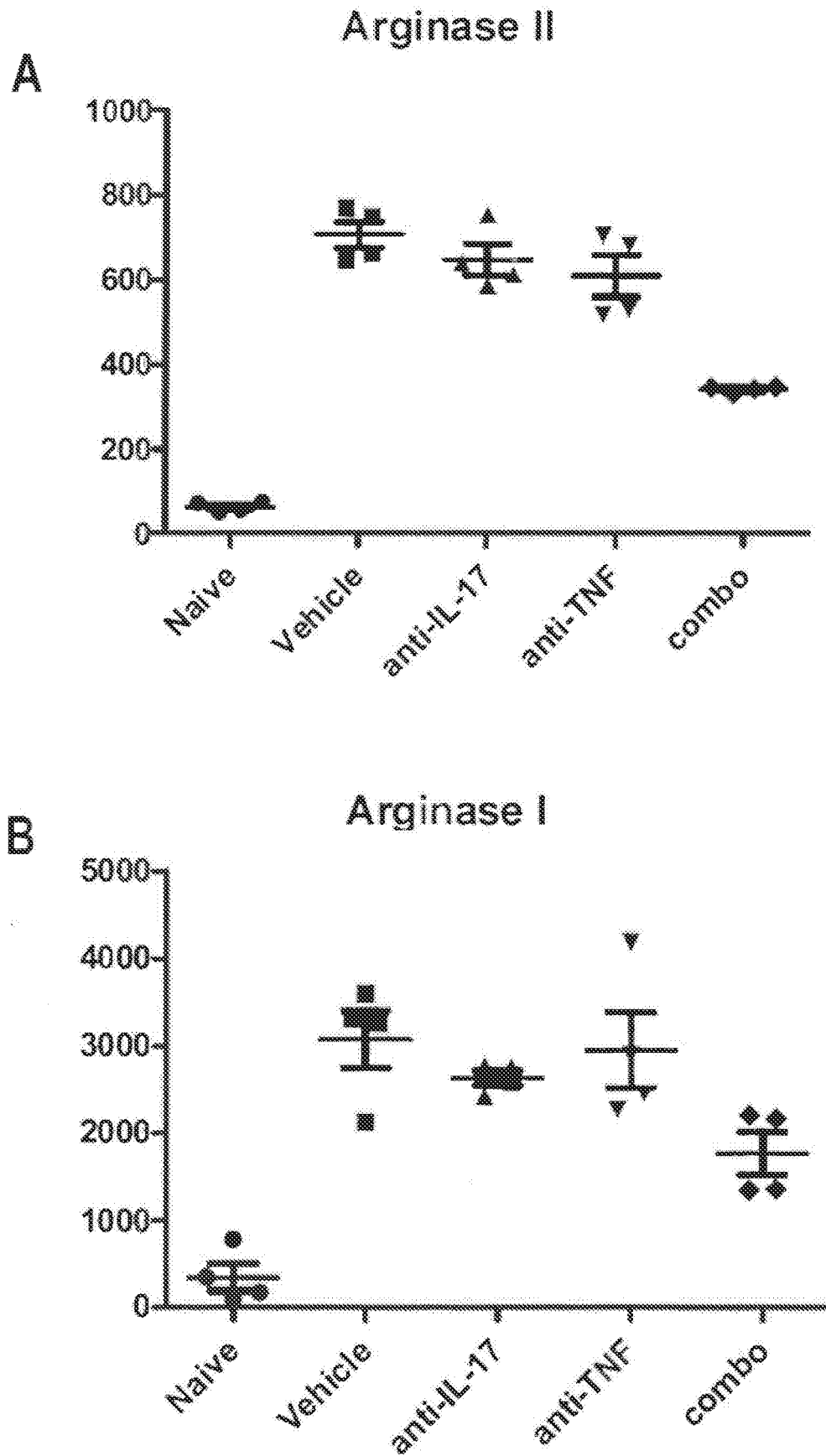
E



Efficacy measured by mean arthritic score (MAS) as well as by microOCT analysis of the tarsal bone and histological analysis of inflammation, bone and cartilage loss

Figure 2



**Fig. 3A-B**

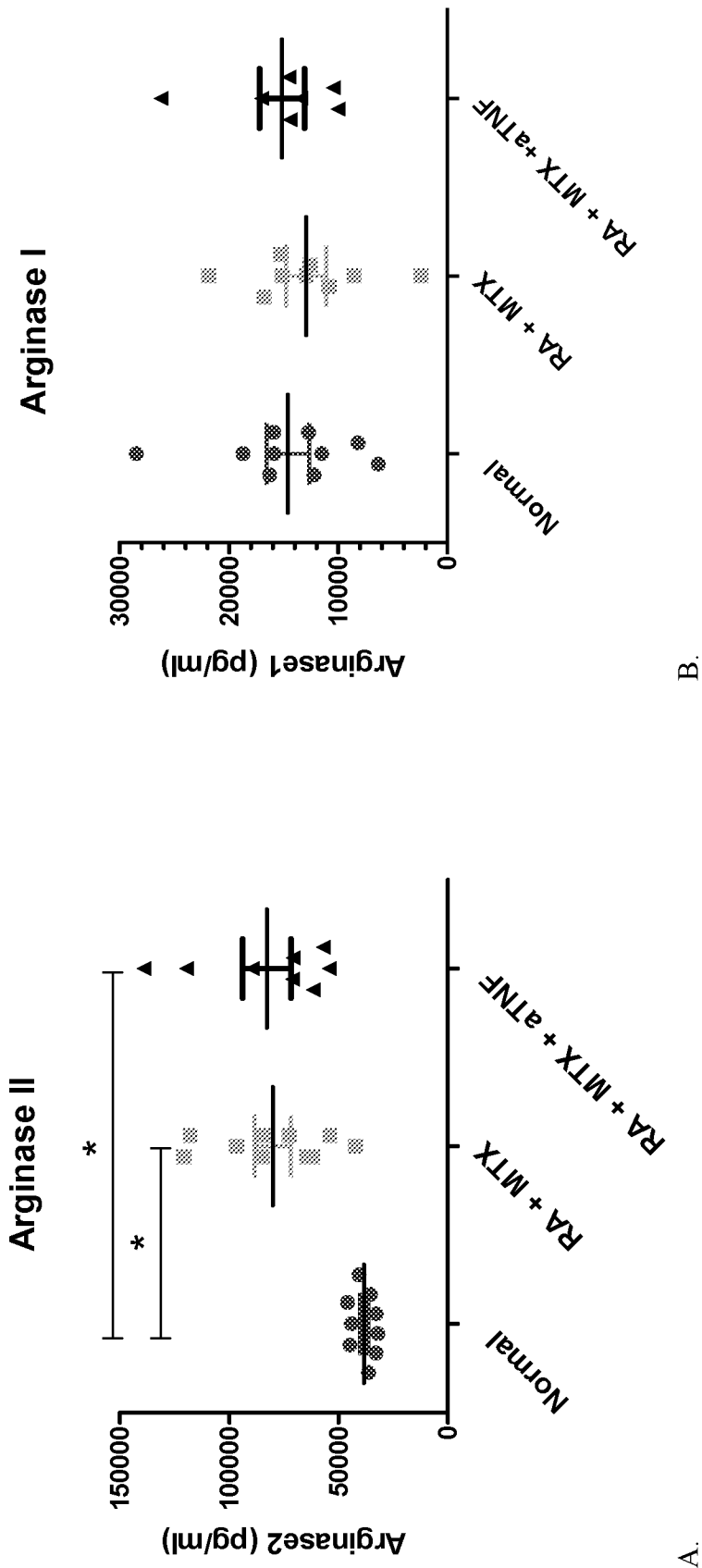


Figure 4

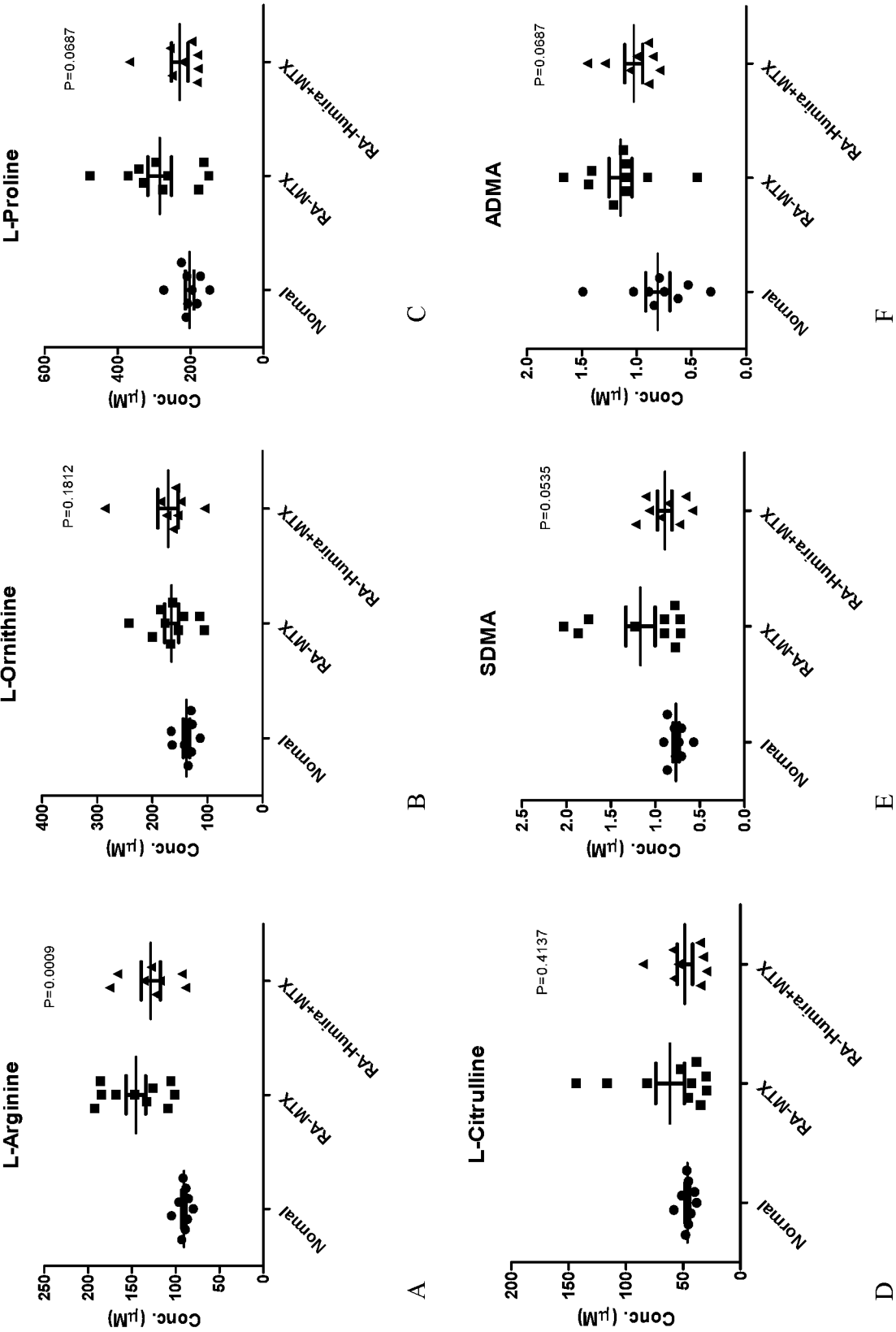


Figure 5

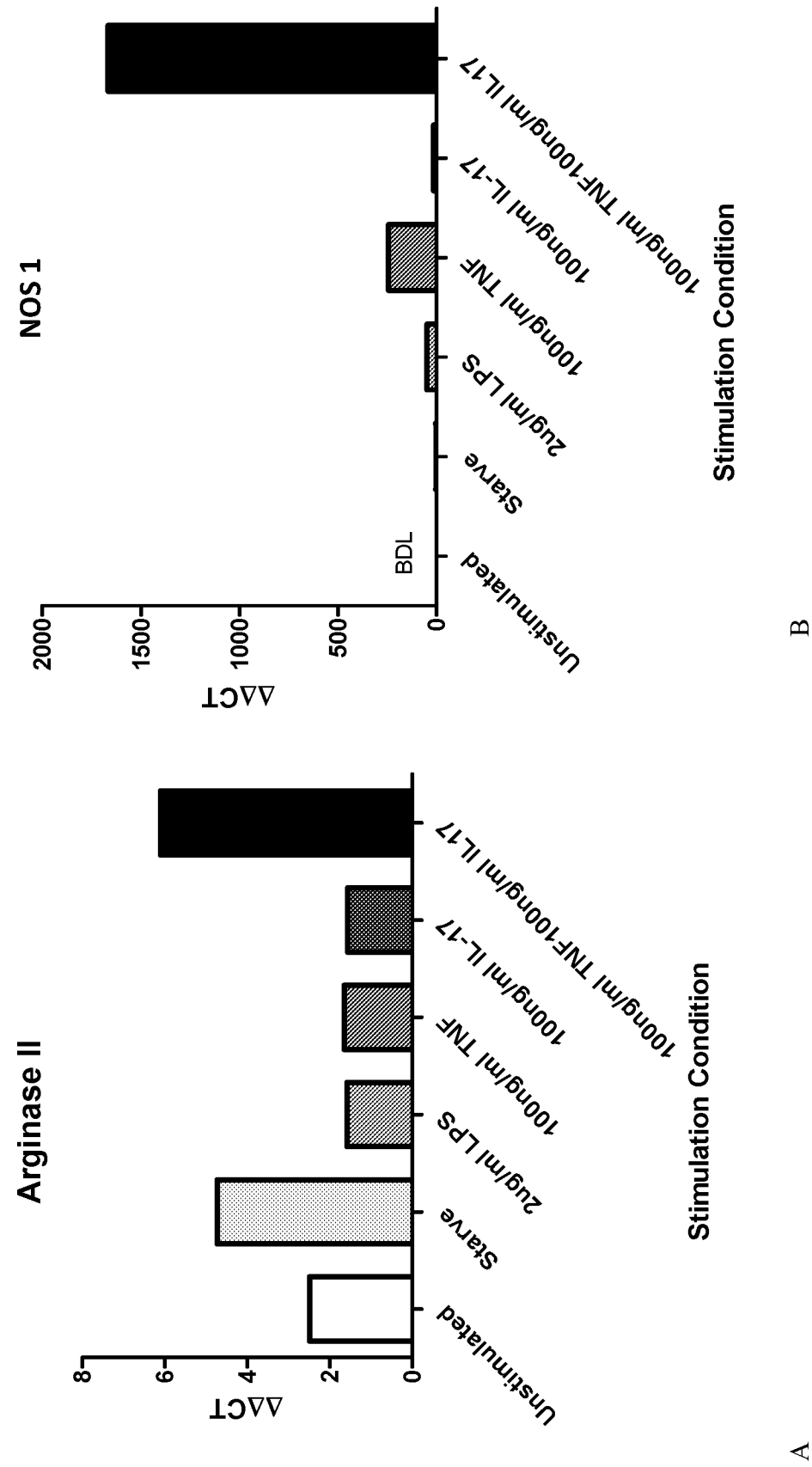
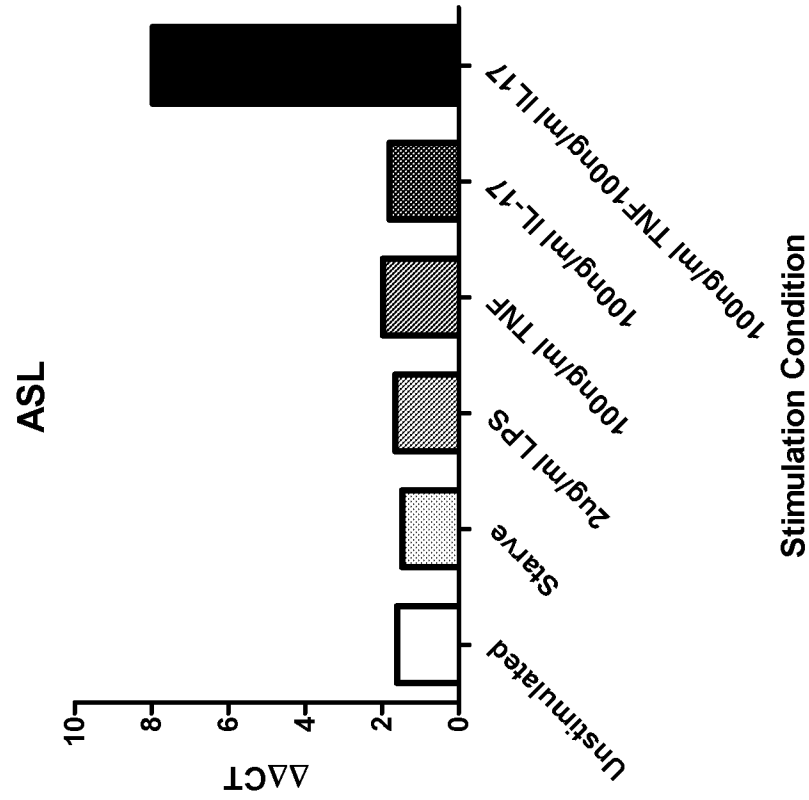
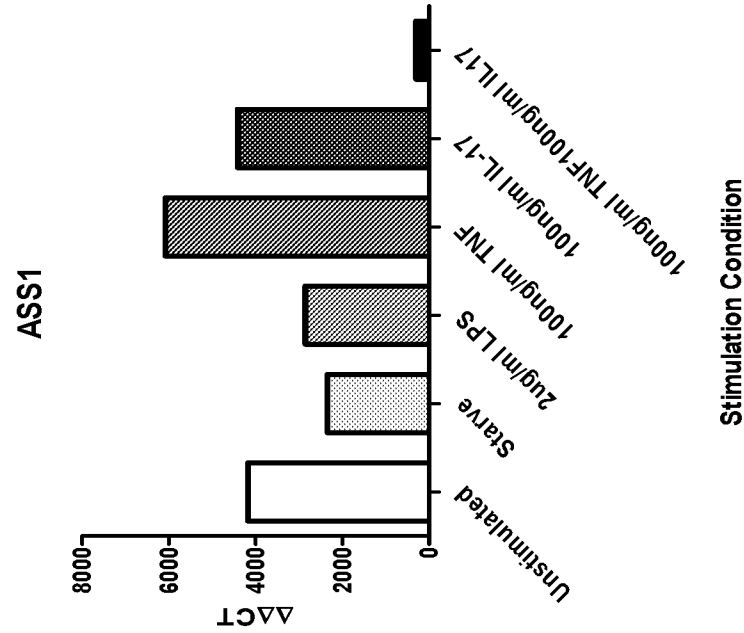


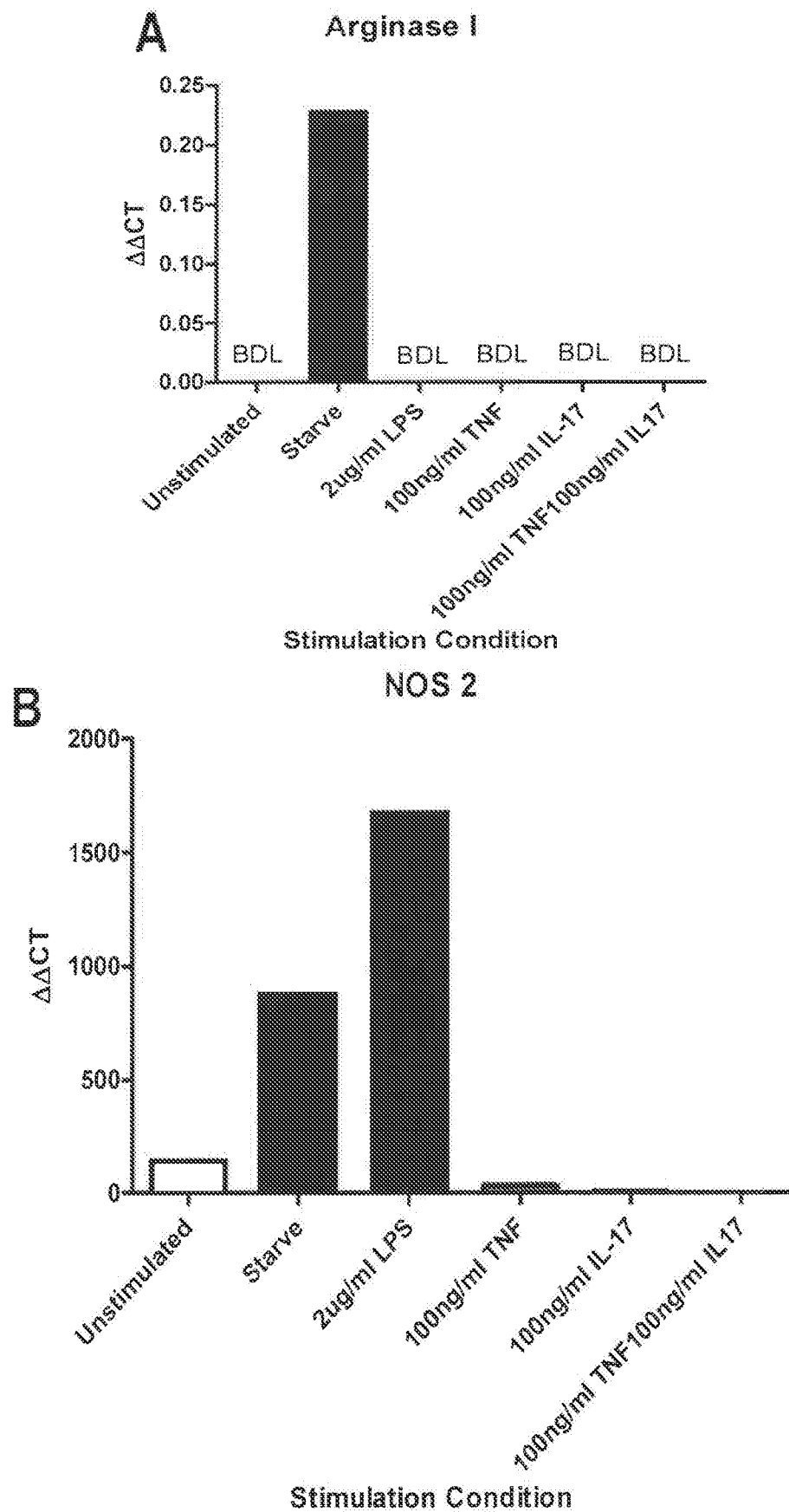
Figure 6



D

C

Figure 6

**Fig. 7A-B**

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/038552A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/564
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, COMPENDEX, EMBASE, INSPEC, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TAKASHI YAMADA ET AL: "Nitrosative modifications of the Ca 2+ release complex and actin underlie arthritis-induced muscle weakness", ANNALS OF THE RHEUMATIC DISEASES, vol. 74, no. 10, 22 May 2014 (2014-05-22), pages 1907-1914, XP055307987, GB	23,26-31
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Further documents are listed in the continuation of Box C.



See patent family annex.

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Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2016/038552

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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X	BELOGOLOVYKH L A ET AL: "Nitroxide synthase activities at different stages of adjuvant arthritis", BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE, KLUWER ACADEMIC PUBLISHERS-PLENUM PUBLISHERS, NE, vol. 143, no. 5, 1 May 2007 (2007-05-01), pages 638-641, XP019548513, ISSN: 1573-8221, DOI: 10.1007/S10517-007-0203-Y	23
Y	abstract Paragraph bridging columns 1 and 2 on page 639 page 640, column 2, paragraphs 1,2	19-21, 32-34
X	US 2009/298912 A1 (BERKOWITZ DAN E [US] ET AL) 3 December 2009 (2009-12-03) paragraphs [0095], [0109]	23-25
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X	CORRALIZA INES ET AL: "Increased expression of arginase II in patients with different forms of arthritis. Implications of the regulation of nitric oxide", JOURNAL OF RHEUMATOLOGY, JOURNAL OF RHEUMATOLOGY PUBLISHING COMPANY, CA, vol. 29, no. 11, 1 November 2002 (2002-11-01), pages 2261-2265, XP009191983, ISSN: 0315-162X cited in the application	23
Y	abstract page 2262, column 1, paragraph 3 - page 2263, column 1, paragraph 2	13-21, 26-34
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Information on patent family members

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